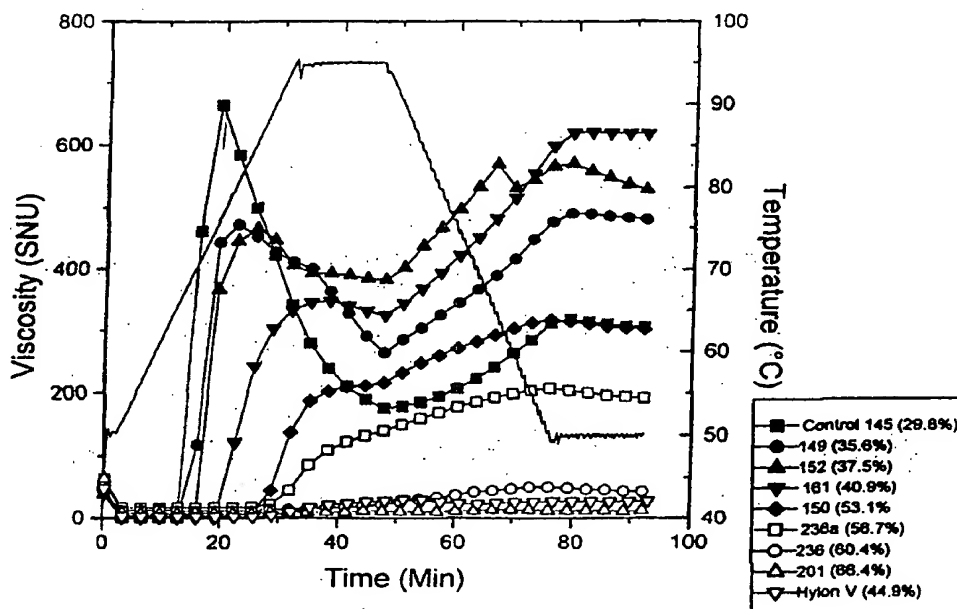




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/82, 15/54, C08B 30/04, A01H 5/00		A2	(11) International Publication Number: WO 96/34968
(21) International Application Number: PCT/GB96/01075		(43) International Publication Date: 7 November 1996 (07.11.96)	
(22) International Filing Date: 3 May 1996 (03.05.96)		[GB/GB]; 46 Castle Street, Wellingborough, Northamptonshire NN8 1LW (GB).	
(30) Priority Data: 9509229.2 5 May 1995 (05.05.95) GB 9607409.1 10 April 1996 (10.04.96) GB		(74) Agent: KEITH W. NASH & CO. ; 90-92 Regent Street, Cambridge CB2 1DP (GB).	
(71) Applicant (for all designated States except US): NATIONAL STARCH AND CHEMICAL INVESTMENT HOLDING CORPORATION [US/US]; Suite 27, 501 Silverside Road, Wilmington, DE 19809 (US).		(81) Designated States: AU, BR, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(72) Inventors; and (75) Inventors/Applicants (for US only): COOKE, David [GB/GB]; 2 Hunts Path, Oakley, Bedfordshire MK43 7SR (GB). DEBET, Martine [FR/GB]; 12 Bentley Close, Rectory Farm, Northampton NN3 5JS (GB). GIDLEY, Michael, John [GB/GB]; 45 Holmes Avenue, Raunds, Northamptonshire NN9 6SZ (GB). JOBLING, Stephen, Alan [GB/GB]; 19 Burwell Road, Eaton Socon, Huntingdon PE19 3QQ (GB). SAFFORD, Richard [GB/GB]; 10 Furness Close, Bedford, Bedfordshire MK41 8RN (GB). SIDEBOTTOM, Christopher, Michael [GB/GB]; 35 Waterloo Road, Bedford, Bedfordshire MK40 3PQ (GB). WESTCOTT, Roger, John		Published <i>Without international search report and to be republished upon receipt of that report.</i>	

(54) Title: IMPROVEMENTS IN OR RELATING TO PLANT STARCH COMPOSITION



(57) Abstract

Disclosed is a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants, or a functional equivalent thereof, together with, inter alia, a corresponding polypeptide, a method of altering the characteristics of a plant, a plant having altered characteristics; and starch, particularly starch obtained from a potato plant, having novel properties.

Title: Improvements in or Relating to Plant Starch Composition

Field of the Invention

This invention relates to novel nucleotide sequences, polypeptides encoded thereby, vectors and host cells and host organisms comprising one or more of the novel sequences, and to a method of altering one or more characteristics of an organism. The invention also relates to starch having novel properties and to uses thereof.

Background of the Invention

Starch is the major form of carbon reserve in plants, constituting 50% or more of the dry weight of many storage organs - e.g. tubers, seeds of cereals. Starch is used in numerous food and industrial applications. In many cases, however, it is necessary to modify the native starches, via chemical or physical means, in order to produce distinct properties to suit particular applications. It would be highly desirable to be able to produce starches with the required properties directly in the plant, thereby removing the need for additional modification. To achieve this via genetic engineering requires knowledge of the metabolic pathway of starch biosynthesis. This includes characterisation of genes and encoded gene products which catalyse the synthesis of starch. Knowledge about the regulation of starch biosynthesis raises the possibility of "re-programming" biosynthetic pathways to create starches with novel properties that could have new commercial applications.

The commercially useful properties of starch derive from the ability of the native granular form to swell and absorb water upon suitable treatment. Usually heat is required to cause granules to swell in a process known as gelatinisation, which has been defined (W A Atwell *et al*, Cereal Foods World 33, 306-311, 1988) as "... *the collapse (disruption) of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilisation. The point of initial gelatinisation and the range over which it occurs is governed by starch concentration, method of observation, granule type, and heterogeneities within the granule population under observation*". A number of techniques are available

cited above) as "...a process which occurs when the molecules comprising gelatinised starch begin to reassociate in an ordered structure...". At 50°C, it is primarily the amylose component which reassociates, as indicated by the increase in viscoamylograph viscosity for starch from normal maize (21.6% amylose) compared with starch from waxy maize (1.1% amylose) as shown in Figure 2. Figure 2 is a viscoamylograph of 10%w/w starch suspensions from waxy maize (solid line), conventional maize (dots and dashes), high amylose variety (hylon 5, dotted line) and a very high amylose variety (hylon 7, crosses). The temperature profile is also shown by a solid line, as in Figure 1. The extent of viscosity increase in the viscoamylograph on cooling and holding at 50°C depends on the amount of amylose which is able to reassociate due to its exudation from starch granules during the gelatinisation and pasting processes. A characteristic of amylose-rich starches from maize plants is that very little amylose is exuded from granules by gelatinisation and pasting up to 95°C, probably due to the restricted swelling of the granules. This is illustrated in Figure 2 which shows low viscosities for a high amylose (44.9%) starch (Hylon 5) from maize during gelatinisation and pasting at 95°C and little increase in viscosity on cooling and holding at 50°C. This effect is more extreme for a higher amylose content (58%, as in Hylon 7), which shows even lower viscosities in the viscoamylograph test (Figure 2). For commercially-available high amylose starches (currently available from maize plants, such as those described above), processing at greater than 100°C is usually necessary in order to generate the benefits of high amylose contents with respect to increased rates and strengths of reassociation, but use of such high temperatures is energetically unfavourable and costly. Accordingly, there is an unmet need for starches of high amylose content which can be processed below 100°C and still show enhanced levels of reassociation, as indicated for example by viscoamylograph measurements.

The properties of potato starch are useful in a variety of both food and non-food (paper, textiles, adhesives etc.) applications. However, for many applications, properties are not optimum and various chemical and physical modifications well known in the art are undertaken in order to improve useful properties. Two types of property manipulation which would be of use are: the controlled alteration of gelatinisation and pasting temperatures; and starches which suffer less granular fragmentation during pasting than

between the two classes. One general distinction of note would appear to be the presence, in class A SBE molecules, of a flexible N-terminal domain, which is not found in class B molecules. The distinctions noted by Burton *et al.* are relied on herein to define class A and class B SBE molecules, which terms are to be interpreted accordingly.

However in potato, only one isoform of the SBE molecule (belonging to class B) has thus far been reported and only one gene cloned (Blennow & Johansson, 1991 *Phytochem.* 30, 437-444, and Koßmann *et al.*, 1991 *Mol. Gen. Genet.* 230, 39-44). Further, published attempts to modify the properties of starch in potato plants (by preventing expression of the single known SBE) have generally not succeeded (e.g. Müller-Rober & Koßmann 1994 *Plant Cell and Environment* 17, 601-613).

Summary of the Invention

In a first aspect the invention provides a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants.

Preferably the nucleotide sequence encodes a polypeptide comprising an effective portion of the amino acid sequence shown in Figure 5 (excluding the sequence MNKRIDL, which does not represent part of the SBE molecule), or a functional equivalent thereof (which term is discussed below). The amino acid sequence shown in Figure 5 (Seq ID No. 15) includes a leader sequence which directs the polypeptide, when synthesised in potato cells, to the amyloplast. Those skilled in the art will recognise that the leader sequence is removed to produce a mature enzyme and that the leader sequence is therefore not essential for enzyme activity. Accordingly, an "effective portion" of the polypeptide is one which possesses sufficient SBE activity to complement the branching enzyme mutation in *E. coli* KV 832 cells (described below) and which is active when expressed in *E. coli* in the phosphorylation stimulation assay. An example of an incomplete polypeptide which nevertheless constitutes an "effective portion" is the mature enzyme lacking the leader sequence. By analogy with the pea class A SBE sequence, the potato class A sequence shown in Figure 5 probably possesses a leader sequence of about 48 amino acid residues, such that the N terminal amino acid sequence is thought to commence around the glutamic acid residue (E) at position 49 (EKSSYN... etc.). Those skilled in the art will appreciate

sequence. Accordingly, functionally equivalent sequences will also include those sequences which can hybridise, under stringent hybridisation conditions, to the sequence of the invention (rather than the complement thereof). Such "antisense" equivalents will preferably possess at least 85%, more preferably at least 90%, and most preferably 95% sequence homology with the complement of the sequence of the invention as exemplified by nucleotides 289 to 2790 of the DNA sequence shown in Figure 5. Particular functional equivalents are shown, for example, in Figures 8 and 10 (if one disregards the various frameshift mutations noted therein).

The invention also provides vectors, particularly expression vectors, comprising the nucleotide sequence of the invention. The vector will typically comprise a promoter and one or more regulatory signals of the type well known to those skilled in the art. The invention also includes provision of cells transformed (which term encompasses transduction and transfection) with a vector comprising the nucleotide sequence of the invention.

The invention further provides a class A SBE polypeptide, obtainable from potato plants. In particular the invention provides the polypeptide in substantially pure form, especially in a form free from other plant-derived (especially potato plant-derived) components, which can be readily accomplished by expression of the relevant nucleotide sequence in a suitable non-plant host (such as any one of the yeast strains routinely used for expression purposes, e.g. *Pichia spp.* or *Saccharomyces spp.*). Typically the enzyme will substantially comprise the sequence of amino acid residues 49 to 882 shown in Figure 5 (disregarding the sequence MNKRIDL, which is not part of the enzyme), or a functional equivalent thereof. The polypeptide of the invention may be used in a method of modifying starch *in vitro*, comprising treating starch under suitable conditions (e.g. appropriate temperature, pH, etc) with an effective amount of the polypeptide according to the invention.

The term "functional equivalent", as applied herein to amino acid sequences, is intended to encompass amino acid sequences substantially similar to that shown in Figure 5, such that the polypeptide possesses sufficient activity to complement the branching enzyme mutation in *E. coli* KV 832 cells (described below) and which is active in *E. coli* in the

at least one third of the full length sequence, but by simple trial and error other fragments (smaller or larger) may be found which are functional in altering the characteristics of the plant.

Thus, in a further aspect the invention provides a method of altering the characteristics of a plant, comprising introducing into the plant an effective portion of the sequence of the invention operably linked to a suitable promoter active in the plant. Conveniently the sequence will be linked in the anti-sense orientation to the promoter. Preferably the plant is a potato plant. Conveniently, the characteristic altered relates to the starch content and/or starch composition of the plant (i.e. amount and/or type of starch present in the plant). Preferably the method of altering the characteristics of the plant will also comprise the introduction of one or more further sequences, in addition to an effective portion of the sequence of the invention. The introduced sequence of the invention and the one or more further sequences (which may be sense or antisense sequences) may be operably linked to a single promoter (which would ensure both sequences were transcribed at essentially the same time), or may be operably linked to separate promoters (which may be necessary for optimal expression). Where separate promoters are employed they may be identical to each other or different. Suitable promoters are well known to those skilled in the art and include both constitutive and inducible types. Examples include the CaMV 35S promoter (e.g. single or tandem repeat) and the patatin promoter. Advantageously the promoter will be tissue-specific. Desirably the promoter will cause expression of the operably linked sequence at substantial levels only in the tissue of the plant where starch synthesis and/or starch storage mainly occurs. Thus, for example, where the sequence is introduced into a potato plant, the operably linked promoter may be tuber-specific, such as the patatin promoter.

Desirably, for example, the method will also comprise the introduction of an effective portion of a sequence encoding a class B SBE, operably linked in the antisense orientation to a suitable promoter active in the plant. Desirably the further sequence will comprise an effective portion of the sequence encoding the potato class B SBE molecule. Conveniently the further sequence will comprise an effective portion of the sequence described by Blennow & Johansson (1991 *Phytochem.* 30, 437-444) or that disclosed in

chips (amylose generally being used as a coating to prevent fat uptake), and to prepare mashed potato (especially "instant" mashed potato) having particular characteristics.

In particular relation to potato plants, the invention provides a potato plant or part thereof which, in its wild type possesses an effective SBE A gene, but which plant has been altered such that there is no effective expression of an SBE A polypeptide within the cells of at least part of the plant. The plant may have been altered by the method defined above, or may have been selected by conventional breeding to be deleted for the class A SBE gene, presence or absence of which can be readily determined by screening samples of the plants with a nucleic acid probe or antibody specific for the potato class A gene or gene product respectively.

The invention also provides starch extracted from a plant altered by the method defined above, or the progeny of such a plant, the starch having altered properties compared to starch extracted from equivalent, but unaltered, plants. The invention further provides a method of making altered starch, comprising altering a plant by the method defined above and extracting therefrom starch having altered properties compared to starch extracted from equivalent, but unaltered, plants. Use of nucleotide sequences in accordance with the invention has allowed the present inventors to produce potato starches having a wide variety of novel properties.

In particular the invention provides the following: a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated endotherm peak temperature as judged by DSC, compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated viscosity onset temperature (conveniently elevated by 10 - 25°C) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has a decreased peak viscosity (conveniently decreased by 240 - 700SNUs) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method

phase (step 5) and has a set-back viscosity of 303 SNU's or less as judged by viscoamylograph according to the protocol defined below.

For the purposes of the present invention, viscoamylograph conditions are understood to pertain to analysis of a 10% (w/w) aqueous suspension of starch at atmospheric pressure, using a Newport Scientific Rapid Visco Analyser with a heating profile of: holding at 50°C for 2 minutes (step 1), heating from 50 to 95°C at a rate of 1.5°C per minute (step 2), holding at 95°C for 15 minutes (step 3), cooling from 95 to 50°C at a rate of 1.5°C per minute (step 4), and then holding at 50°C for 15 minutes (step 5). Peak viscosity may be defined for present purposes as the maximum viscosity attained during the heating phase (step 2) or the holding phase (step 3) of the viscoamylograph. Pasting viscosity may be defined as the viscosity attained by the starch suspensions at the end of the holding phase (step 3) of the viscoamylograph. Set-back viscosity may be defined as the viscosity of the starch suspension at the end of step 5 of the viscoamylograph.

In yet another aspect the invention provides starch from a potato plant having an apparent amylose content (% w/w) of at least 35%, as judged by iodometric assay according to the method described by Morrison & Laignelet (1983 J. Cereal Science 1, 9-20). Preferably the starch will have an amylose content of at least 40%, more preferably at least 50%, and most preferably at least 66%. Starch obtained directly from a potato plant and having such properties has not hitherto been produced. Indeed, as a result of the present invention, it is now possible to generate *in vivo* potato starch which has some properties analogous to the very high amylose starches (e.g. Hylon 7) obtainable from maize.

Starches with high (at least 35%) amylose contents find commercial application as, amongst other reasons, the amylose component of starch reassociates more strongly and rapidly than the amylopectin component during retrogradation processes. This may result, for example, in pastes with higher viscosities, gels of greater cohesion, or films of greater strength for starches with high (at least 35%) compared with normal (less than 35%) amylose contents. Alternatively, starches may be obtained with very high amylose contents, such that the granule structure is substantially preserved during heating, resulting in starch suspensions which demonstrate substantially no increase in viscosity during

Under the heading of "Industrial" applications, the novel starches of the invention may be advantageously employed, for example, in corrugating adhesives, in biodegradable products such as loose fill packaging and foamed shapes, and in the production of glass fibers and textiles.

Those skilled in the art will appreciate that the novel starches of the invention may, if desired, be subjected *in vitro* to conventional enzymatic, physical and/or chemical modification, such as cross-linking, introduction of hydrophobic groups (e.g. octenyl succinic acid, dodecyl succinic acid), or derivatization (e.g. by means of esterification or etherification).

In yet another aspect the invention provides high (35% or more) amylose starches which generate paste viscosities greater than those obtained from high amylose starches from maize plants after processing at temperatures below 100°C. This provides the advantage of more economical starch gelatinisation and pasting treatments through the use of lower processing temperatures than are currently required for high amylose starches from maize plants.

The invention will now be further described by way of illustrative example and with reference to the drawings, of which:

Figure 1 shows a typical viscoamylograph for a 10% w/w suspension of potato starch;

Figure 2 shows viscoamylographs for 10% suspensions of starch from various maize varieties;

Figure 3 is a schematic representation of the cloning strategy used by the present inventors;

Figure 4a shows the amino acid alignment of the C-terminal portion of starch branching enzyme isoforms from various sources: amino acid residues matching the consensus

Examples

Example 1

Cloning of Potato class A SBE

The strategy for cloning the second form of starch branching enzyme from potato is shown in Figure 3. The small arrowheads represent primers used by the inventors in PCR and RACE protocols. The approximate size of the fragments isolated is indicated by the numerals on the right of the Figure. By way of explanation, a comparison of the amino acid sequences of several cloned plant starch branching enzymes (SBE) from maize (class A), pea (class A), maize (class B), rice (class B) and potato (class B), as well as human glycogen branching enzyme, allowed the inventors to identify a region in the carboxy-terminal one third of the protein which is almost completely conserved (GYLNFMGNEFGHPEWIDFPR) (Figure 4a). A multiple alignment of the DNA sequences (human, pea class A, potato class B, maize class B, maize class A and rice class B, respectively) corresponding to this region is shown in Figure 4b and was used to design an oligo which would potentially hybridize to all known plant starch branching enzymes: AATTT(C/T)ATGGGIAA(C/T)GA(A/G)TT(C/T)GG (Seq ID No. 20).

Library PCR

The initial isolation of a partial potato class A SBE cDNA clone was from an amplified potato tuber cDNA library in the λ Zap vector (Stratagene). One half μ L of a potato cDNA library (titre 2.3×10^9 pfu/mL) was used as template in a 50 μ L reaction containing 100 pmol of a 16 fold degenerate POTSBE primer and 25 pmol of a T7 primer (present in the λ Zap vector 3' to the cDNA sequences - see Figure 3), 100 μ M dNTPs, 2.5 U Taq polymerase and the buffer supplied with the Taq polymerase (Stratagene). All components except the enzyme were added to a 0.5 mL microcentrifuge tube, covered with mineral oil and incubated at 94°C for 7 minutes and then held at 55°C, while the Taq polymerase was added and mixed by pipetting. PCR was then performed by incubating for 1 min at 94°C, 1 min at 58°C and 3 minutes at 72°C. for 35 cycles. The PCR products were extracted with phenol/chloroform, ethanol precipitated and resuspended in TE pH 8.0 before cloning into the T/A cloning vector pT7BlueR (Invitrogen).

primer. Excess primers were removed on a Centricon 100 column and cDNA was recovered and precipitated with isopropanol. cDNA was A-tailed in a volume of 20 μ l using 10 units terminal transferase (BRL), 200 μ M dATP for 10 min at 37°C, followed by 5 min at 65°C. The reaction was then diluted to 0.5 ml with TE pH 8 and stored at 4°C as the cDNA pool. cDNA clones were isolated by PCR amplification using the primers $R_0R_1dT_{17}$, R_0 and POTSBE24. The PCR was performed in 50 μ L using a hot start technique: 10 μ L of the cDNA pool was heated to 94°C in water for 5 min with 25 pmol POTSBE24, 25 pmol R_0 and 2.5 pmol of $R_0R_1dT_{17}$ and cooled to 75°C. Five μ L of 10 x PCR buffer (Stratagene), 200 μ M dNTPs and 1.25 units of Taq polymerase were added, the mixture heated at 45°C for 2 min and 72°C for 40 min followed by 35 cycles of 94°C for 45 sec, 50°C for 25 sec, 72°C for 1.5 min and a final incubation at 72°C for 10 min. PCR products were separated by electrophoresis on 1% low melting agarose gels and the smear covering the range 600-800 bp fragments was excised and used in a second PCR amplification with 25 pmol of R_1 and POTSBE25 primers in a 50 μ L reaction (28 cycles of 94°C for 1 min, 50°C 1 min, 72°C 2 min). Products were purified by chloroform extraction and cloned into pT7 Blue. PCR was used to screen the colonies and the longest clones were sequenced.

The first round of RACE only extended the length of the SBE sequence approximately 100 bases, therefore a new A-tailed cDNA library was constructed using the class A SBE specific oligo POTSBE24 (10 pmol) in an attempt to recover longer RACE products. The first and second round PCR reactions were performed using new class A SBE primers (POTSBE 28 and 29 respectively) derived from the new sequence data. Conditions were as before except that the elongation step in the first PCR was for 3 min and the second PCR consisted of 28 cycles at 94 °C for 45 seconds, 55 °C for 25 sec and 72 °C for 1 min 45 sec.

Clones ranging in size from 400 bp to 1.4 kb were isolated and sequenced. The combined sequence of the longest RACE products and cDNA clones predicted a full length gene of about 3150 nucleotides, excluding the poly(A) tail (psbe 2con.seq in Fig. 8).

As the sequence of the 5' half of the gene was compiled from the sequence of several

degree of similarity, identical residues being denoted by the common letter, conservative changes by two dots and neutral changes by a single dot. Dashes indicate gaps introduced to optimise the alignment. The class A SBE protein has 44% identity over the entire length with potato class B SBE, and 56% identity therewith in the central conserved domain (Figure 6), as judged by the "Megalign" program (DNASTAR). However, Figure 7 shows a comparison between potato class A SBE (top row, residues 1-873) and pea class A SBE (bottom row, residues 1-861), from which it can be observed that cloned potato gene is more homologous to the class A pea enzyme, where the identity is 70 % over nearly the entire length, and this increases to 83 % over the central conserved region (starting at IPPP at position ~170). It is clear from this analysis that this cloned potato SBE gene belongs to the class A family of SBE genes.

An *E. coli* culture, containing the plasmid pSJ78 (which directs the expression of a full length potato SBE Class A gene), has been deposited (on 3rd January 1996) under the terms of the Budapest Treaty at The National Collections of Industrial and Marine Bacteria Limited (23 St Machar Drive, Aberdeen, AB2 1RY, United Kingdom), under accession number NCIMB 40781. Plasmid pSJ78 is equivalent to clone 19 described above. It represents a full length SBE A cDNA blunt-end ligated into the vector pBSSKIIP.

Polymorphism of class A SBE genes

Sequence analysis of the other two full length class A SBE genes showed that they contain frameshift mutations and are therefore unable to encode full length proteins and indeed they were unable to complement the branching enzyme deficiency in the KV832 mutant (described below). An alignment of the full length DNA sequences is shown in Figure 8: "10con.seq" (Seq ID No. 12), "19con.seq" (Seq ID No. 14) and "11con.seq" (Seq ID No. 13) represent the sequence of full length clones 10, 19 and 11 obtained by PCR using the PBER1 and PBERT primers (see below), whilst "psbe2con.seq" (Seq ID No. 18) represents the consensus sequence of the RACE clones and cDNA clone 3.2.1. Those nucleotides which differ from the overall consensus sequence (not shown) are shaded. Dashes indicate gaps introduced to optimise the alignment. Apart from the frameshift mutations these clones are highly homologous. It should be noted that the 5' sequence of psbe2con is longer because this is the longest RACE product and it also contains several

A comparison of all the full length sequences is shown in Figure 10. In addition to clones 10, 11 and 19 are shown the sequences of a *Bgl* II - *Xho* I product cloned directly into the QE32 expression vector ("86CON.SEQ", Seq ID No. 16) and the consensus sequence of the directly sequenced PCR products ("pcrsbe2con.seq", Seq ID No. 17). Those nucleotides which differ from the consensus sequence (not shown) are shaded. Dashes indicate gaps introduced to optimise the alignment. There are 11 nucleotide differences predicted to be present in the mRNA population, which are indicated by asterisks above and below the sequence. The other differences are probably PCR artefacts or possibly sequencing errors.

Complementation of a branching enzyme deficient *E. coli* mutant

To determine if the isolated SBE gene encodes an active protein i.e. one that has branching enzyme activity, a complementation test was performed in the *E. coli* strain KV832. This strain is unable to make bacterial glycogen as the gene for the glycogen branching enzyme has been deleted (Keil *et al.*, 1987 Mol. Gen. Genet. 207, 294-301). When wild type cells are grown in the presence of glucose they synthesise glycogen (a highly branched glucose polymer) which stains a brown colour with iodine, whereas the KV832 cells make only a linear chain glucose polymer which stains blueish green with iodine. To determine if the cloned SBE gene could restore the ability of the KV832 cells to make a branched polymer, the clone pSJ90 (Seq ID No. 19) was used and constructed as below. The construct is a PCR-derived, substantially full length fragment (made using primers PBE 2B and PBE 2X, detailed below), which was cut with *Bgl* II and *Xho* I and cloned into the *Bam*H I / *Sal* I sites of the His-tag expression vector pQE32 (Qiagen). This clone, pSJ86, was sequenced and found to have a frameshift mutation of two bases in the 5' half of the gene. This frameshift was removed by digestion with *Nsi* I and *Sna*B I and replaced with the corresponding fragment from a Taq-generated PCR clone to produce the plasmid pSJ90 (sequence shown in Figure 12; the first 10 amino acids are derived from the expression vector). The polypeptide encoded by pSJ90 would be predicted to correspond to amino acids 46-882 of the full SBE coding sequence. The construct pSJ90 was transformed into the branching enzyme deficient KV832 cells and transformants were grown on solid PYG medium (0.85% KH_2PO_4 , 1.1% K_2HPO_4 , 0.6% yeast extract) containing 1.0% glucose. To test for complementation, a loop of cells was

phosphorylation stimulation assay and that the cloned gene does indeed code for a protein with branching enzyme activity.

Oligonucleotides

The following synthetic oligonucleotides (Seq ID No.s 1-11 respectively) were used:

R ₀ R _i dT ₁₇	AAGGATCCGTCGACATCGATAATACGACTCACTATAGGGA(T) ₁₇
R ₀	AAGGATCCGTCGACATC
R _i	GACATCGATAATACGAC
POTSBE24	CATCCAACCACCATCTCGCA
POTSBE25	TTGAGAGAAGATACCTAAGT
POTSBE28	ATGTTTCAGTCCATCTAAAGT
POTSBE29	AGAACAACAATTCCTAGCTC
PBER 1	GGGGCCTTGAAGTCAGCAAT
PBERT	CGTCCCAGCATTTCGACATAA
PBE 2B	CTTGATCCTTGAAGTCAGCAATTTG
PBE 2X	TAACTCGAGCAACGCGATCACAAGTTCGT

Example 2

Production of Transgenic Plants

Construction of plant transformation vectors with antisense starch branching enzyme genes

A 1200 bp *Sac* I - *Xho* I fragment, encoding approximately the -COOH half of the potato class A SBE (isolated from the rescued λ Zap clone 3.2.1), was cloned into the *Sac* I - *Sal* I sites of the plant transformation vector pSJ29 to create plasmid pSJ64, which is illustrated schematically in Figure 11. In the figure, the black line represents the DNA sequence. The broken line represents the bacterial plasmid backbone (containing the origin of replication and bacterial selection marker), which is not shown in full. The filled triangles on the line denote the T-DNA borders (RB = right border, LB = left border). Relevant restriction sites are shown above the black line, with the approximate distances (in kilobases) between the sites (marked by an asterisk) given by the numerals below the

Transformation was confirmed in all cases by production of a DNA fragment from the antisense gene after PCR in the presence of appropriate primers and a crude extract of genomic DNA from each regenerated shoot.

Characterisation of starch from potato plants

Starch was extracted from plants as follows: potato tubers were homogenised in water for 2 minutes in a Waring blender operating at high speed. The homogenate was washed and filtered (initially through 2mm, then through 1mm filters) using about 4 litres of water per 100gms of tubers (6 extractions). Washed starch granules were finally extracted with acetone and air dried.

Starch extracted from singly transformed potato plants (class A/SBE II antisense, or class B/SBE I antisense), or from double transformants (class A/SBE II and class B/SBE I antisense), or from untransformed control plants, was partially characterised. The results are shown in Table 1. The table shows the amount of SBE activity (units/gram tissue) in tubers from each transformed plant. The endotherm peak temperature (°C) of starch extracted from several plants was determined by DSC, and the onset temperature (°C) of pasting was determined by reference to a viscoamylograph ("RVA"), as described in WO 95/26407. The viscoamylograph profile was as follows: step 1 - 50°C for 2 minutes; step 2 - increase in temperature from 50°C to 95°C at a rate of 1.5°C per minute; step 3 - holding at 95°C for 15 minutes; step 4 - cooling from 95°C to 50°C at a rate of 1.5°C per minute; and finally, step 5 - holding at 50°C for 15 minutes. Table 1 shows the peak, pasting and set-back viscosities in stirring number units (SNUs), which is a measure of the amount of torque required to stir the suspensions. Peak viscosity may be defined for present purposes as the maximum viscosity attained during the heating phase (step 2) or the holding phase (step 3) of the viscoamylograph. Pasting viscosity may be defined as the viscosity attained by the starch suspensions at the end of the holding phase (step 3) of the viscoamylograph. Set-back viscosity may be defined as the viscosity of the starch suspension at the end of step 5 of the viscoamylograph.

A determination of apparent amylose content (% w/w) was also performed, using the iodometric assay method of Morrison & Laignelet (1983 *J. Cereal Sci.* 1, 9-20). The

Table 1

Sample description		Sample number	Tuber SBE activity (µg starch)	DSC		Viscosity/ograph				(RVA)		Apparent amylose content (% whw)	Phosphorus content (mg/100g)
				Peak temperature (°C)	Onset temperature (°C)	Peak viscosity (SNU)	Pasting viscosity (SNU)	Set-back viscosity (SNU)					
Untransformed control		146	7.6	65.8	65.5	545	161	260			31.2	66	
		243	22.2	nd	62.6	761	135	241			28.1		
AS-Class A SBE		152	12.7	68.5	70.9	467	360	529			37.5	68	
		249	13.9	nd	70.0	407	434	516			36.5		
AS-Class B SBE (17) (control)		145	0.7	68.9	68.8	669	177	305			29.8	111	
AS-Class B SBE (17) + AS-Class A SBE		150	0.6	74.0	68.0	214	214	303			53.1	196	
		181	0.5	73.0	76.6	349	324	618			40.9	206	
AS-Class B SBE (19) (control)		144	1.6	64.5	64.7	714	154	258			20.0	97	
AS-Class B SBE (18) + AS-Class A SBE		149	3.0	68.5	69.9	474	267	482			35.6	127	
AS-Class B SBE (15) (control)		172	0.22	nd	65.4	707	167	260			28.8	130	
AS-Class B SBE (13) + AS-Class A SBE		201	0.10	nd	>95	no peak	12	13			66.4	210	
		206a	0.10	nd	>95	no peak	15	17			64.1		
		208	0.30	72.8-80.5	>95	no peak	14	19			62.8	240	
		202	0.02	nd	69.4	no peak	172	245			57.9		
		212	1.40	nd	78.0	308	266	541			48.5		
		220	1.40	nd	75.8	355	345	593			44.1		
AS-Class B SBE (12) (control)		170	0.2	nd	66.5	766	202	303			27.6		
AS-Class B SBE (12) + AS-Class A SBE		236	0.7	nd	95.0	no peak	23	14			60.4		
		236a	0.9	nd	91.2	no peak	139	192			56.7		
		230a	0.6	nd	77.6	244	239	450			46.2		

RVA profile

50°C (2 min), 50-95°C (1.5°C/min), 95°C (15 min), 95-50°C (1.5°C/min), 50°C (15 min)

Pasting viscosity (47 min)

at end of 50°C (2min), 50-95°C (1.5°C/min), 95°C (15 min)

Set back viscosity (92 min)

at end of profile

SBE

Starch Branching Enzyme

Instrument "Stirring Number Units" (arbitrary units)

nd

not determined

29/2

Viscoamylograph			Apparent amylose content (% w/w)	Phosphorus content (mg/100g)
Peak viscosity (SNU)	Pasting viscosity (SNU)	Set-back viscosity (SNU)		
545	161	260	31.2	68
761	135	241	29.1	
467	380	529	37.5	89
497	434	518	38.5	
669	177	305	29.8	111
214	214	303	53.1	198
349	324	618	40.9	206
714	154	258	29.0	97
474	267	482	35.6	127

SUBSTITUTE SHEET (RULE 26)

29/4

707	167	290	28.8	130
no peak	12	13	66.4	210
no peak	15	17	64.1	
no peak	14	19	62.8	240
no peak	172	245	57.9	
308	296	541	49.5	
355	345	593	44.1	
768	202	303	27.8	
no peak	23	14	60.4	
no peak	139	192	56.7	
244	239	450	48.2	

SUBSTITUTE SHEET (RULE 26)

149 (35.6% amylose): shaded triangle, pointing upwards - plant 152 (37.5%); shaded triangle, pointing downwards - plant 161 (40.9%); shaded diamond - plant 150 (53.1%); unshaded box - plant 236a (56.7%); unshaded circle - plant 236 (60.4%); unshaded triangle, pointing upwards - plant 201 (66.4%); unshaded triangle, pointing downwards - Hylon V starch, from maize (44.9 % amylose). The thin line denotes the heating profile.

With increasing amylose content, peak viscosities during processing to 95°C decrease, and the drop in viscosity from the peak until the end of the holding period at 95°C also generally decreases (indeed, for some of the starch samples there is an increase in viscosity during this period). Both of these results are indicative of reduced granule fragmentation, and hence *increased* granule stability during pasting. This property has not previously been available in potato starch without extensive prior chemical or physical modification. For applications where a maximal viscosity after processing to 95°C is desirable (i.e. corresponding to the viscosity after 47 minutes in the viscoamylograph test), starch from plant #152 would be selected as starches with both lower (Controls, #149) and higher (#161, #150) amylose contents have lower viscosities following this gelatinisation and pasting regime (Figure 13 and Table 1). It is believed that the viscosity at this stage is determined by a combination of the extent of granule swelling and the resistance of swollen granules to mechanical fragmentation. For any desired viscosity behaviour, one skilled in the art would select a potato starch from a range containing different amylose contents produced according to the invention by performing suitable standard viscosity tests.

Upon cooling pastes from 95°C to 50°C, potato starches from most plants transformed in accordance with the invention showed an increase in viscoamylograph viscosity as expected for partial reassociation of amylose. Starches from plants #149, 152 and 161 all show viscosities at 50°C significantly in excess of those for starches from control plants (Figure 13 and Table 1). This contrasts with the effect of elevated amylose contents in starches from maize plants (Figure 2) which show very low viscosities throughout the viscoamylograph test. Of particular note is the fact that, for similar amylose contents, starch from potato plant 150 (53% amylose) shows markedly increased viscosity compared with Hylon 5 starch (44.9% amylose) as illustrated in Figure 13. This demonstrates that

201, 208 and 208a did not display a clearly defined pasting viscosity peak during the heating phase. Microscopic analysis confirmed that the starch granule structure underwent only minor swelling during the experimental heating process. This property may well be particularly useful in certain applications, as will be apparent to those skilled in the art.

Some re-grown plants have so far been found to increase still further the apparent amylose content of starch extracted therefrom. Such increases may be due to:-

- i) Growth and development of the first generation transformed plants may have been affected to some degree by the exogenous growth hormones present in the tissue culture system, which exogenous hormones were not present during growth of the second generation plants; and
- ii) Subsequent generations were grown under field conditions, which may allow for attainment of greater maturity than growth under laboratory conditions, it being generally held that amylose content of potato starch increases with maturity of the potato tuber.

Accordingly, it should be possible to obtain potato plants giving rise to tubers with starch having an amylose content in excess of the 66% level so far attained, simply by analysing a greater number of transformed plants and/or by re-growing transgenic plants through one or more generations under field conditions.

Table 1 shows that another characteristic of starch which is affected by the presence of anti-sense sequences to SBE is the phosphorus content. Starch from untransformed control plants had a phosphorus content of about 60-70mg/100gram dry weight (as determined according to the AOAC Official Methods of Analysis, 15th Edition, Method 948.09 "Phosphorus in Flour"). Introduction into the plant of an anti-sense SBE B sequence was found to cause a modest increase (about two-fold) in phosphorus content, which is in agreement with the previous findings reported at scientific meetings. Similarly, anti-sense to SBE A alone causes only a small rise in phosphorus content relative to untransformed controls. However, use of anti-sense to both SBE A and B in combination results in up to a four-fold increase in phosphorus content, which is far greater than any *in planta* phosphorus content previously demonstrated for potato starch.

This is useful in that, for certain applications, starch must be phosphorylated *in vitro* by

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: National Starch and Chemical Investment
Holding Corporation
- (B) STREET: 501 Silverside Road, Suite 27
- (C) CITY: Wilmington
- (D) STATE: Delaware
- (E) COUNTRY: United States of America
- (F) POSTAL CODE (ZIP): 19809

(ii) TITLE OF INVENTION: Improvements in or Relating to Plant Starch
Composition

(iii) NUMBER OF SEQUENCES: 20

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0. Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AAGGATCCGT CGACATCGAT AATACGACTC ACTATAGGGA TTTTTTTTTT TTTTTT

57

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AAGGATCCGT CGACATC

17

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AGAACAACAA TTCCTAGCTC

20

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGGGCCTTGA ACTCAGCAAT

20

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CGTCCCAGCA TTCGACATAA

20

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CTTGATCCT TGAATCAGC AATTG

26

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TAACTCGAGC AACGCGATCA CAAGTTCGT

29

GAACGCCCCGA CGACCTTAAG TCTTTGATTG ATAAAGCTCA TGAGCTAGGA ATTGTTGTTT 1440
TCATGGACAT TGTTCACAGC CATGCATCAA ATAATACTTT AGATGGACTG AACATGTTTG 1500
ACGGCACAGA TAGTTGTTAC TTTCACCTCG GAGCTCGTGG TTATCATTGG ATGTGGGATT 1560
TCCGCCTCTT TAACTATGGA AACTGGGAGG TACTTAGGTA TCTTCTCTCA AATGCGAGAT 1620
GGTGGTTGGA TGAGTTCAAA TTTGATGGAT TTAGATTTGA TGGTGTGACA TCAATGATGT 1680
GTA CTACCA CGGATTATCG GTGGGATTCA CTGGGA ACTA CGAGGAATAC TTTGGACTCG 1740
CAACTGATGT GGATGCTGTT GTGTATCTGA TGCTGGTCAA CGATCTTATT CATGGGCTTT 1800
TCCCAGATGC AATTACCATT GGTGAAGATG TTAGCGGAAT GCCGACATTT TGTGTTCCCG 1860
TTCAAGATGG GGGTGTGGC TTTGACTATC GGCTGCATAT GGCAATTGCT GATAAATGGA 1920
TTGAGTTGCT CAAGAAACGG GATGAGGATT GGAGAGTGGG TGATATTGTT CATACTGA 1980
CAAATAGAAG ATGGTCGGAA AAGTGTTTT CATACGCTGA AAGTCATGAT CAAGCTCTAG 2040
TCGGTGATAA AACTATAGCA TTCTGGCTGA TGGACAAGGA TATGTATGAT TTTATGGCTC 2100
TGGATAGACC GTCAACATCA TTAATAGATC GTGGGATAGC ATTACACAAG ATGATTAGGC 2160
TTGTA ACTAT GGGATTAGGA GGAGAAGGGT ACCTAAATTT CATGGGAAAT GAATTCGGCC 2220
ACCCTGAGTG GATTGATTTT CCTAGGGCTG AACACACCT CTCTGATGGC TCAGTAATTC 2280
CCAGAAACCA ATTCAGTTAT GATAAATGCA GACGGAGATT TGACCTGGGA GATGCAGAAT 2340
ATTTAAGATA CCGTGGGTTG CAAGAATTTG ACCGGGCTAT GCAGTATCTT GAAGATAAAT 2400
ATGAGTTTAT GACTTCAGAA CACCAGTTCA TATCACGAAA GGATGAAGGA GATAGGATGA 2460
TTGTATTTGA AAAAGGAAAC CTAGTTTTTG TCTTTAATTT TCACTGGACA AAAGGCTATT 2520
CAGACTATCG CATAGGCTGC CTGAAGCCTG GAAAATACAA GGTTGCCTTG GACTCAGATG 2580
ATCCACTTTT TGGTGGCTTC GGGAGAATTG ATCATAATGC CGAATATTTT ACCTTTGAAG 2640
GATGGTATGA TGATCGTCCT CGTTCAATTA TGGTGTATGC ACCTAGTAGA ACAGCAGTGG 2700
TCTATGCACT AGTAGACAAA GAAGAAGAAG AAGAAGAAGA AGTAGCAGTA GTAGAAGAAG 2760
TAGTAGTAGA AGAAGAATGA ACGAACTTGT GATCGCGTTG AAAGATTTGA ACGCCACATA 2820
GAGCTTCTTG ACGTATCTGG CAATATTGCA TTAGTCTTGG CGGAATTTCA TGTGACAACA 2880
GGTTTGCAAT TCTTTCCACT ATTAGTAGTG CAACGATATA CGCAGAGATG AAGTGCTGAA 2940
CAAAAACATA TGTA AAATCG ATGAATTTAT GTCGAATGCT GGGACGATCG AATTCCTGCA 3000
GCC 3003

GAACGCCCCGA	CGACCTTAAG	TCTTCGATTG	ATAAAGCTCA	TGAGCTAGGA	ATTGTTGTTT	1440
TCATGGACAT	CGTTCACAGC	CATGCATCAA	ATAATACTTT	AGATGGACTG	AACATGTTTG	1500
ACGGCACC	GA TAGTTGTTAC	TTTCACTCTG	GAGCTCGTGG	TTATCATTGG	ATGTGGGATT	1560
CCGCCTCTTT	AACTATGGAA	ACTGGGAGGT	ACTTAGGTAT	CTTCTCTCAA	ATGCCGAGATG	1620
GTGGTTGGAT	GAGTTCAAAT	TTGATGGATT	TAGATTTCGAT	GGTGTGACAT	CAATGATGTA	1680
TACTCACCAC	GGATTATCGG	TGGGATTCAC	TGGGAACTAC	GAGGAATACT	TTGGACTCGC	1740
AACTGATGTG	GATGCTGTTG	TGTATCTGAT	GCTGGTCAAC	GATCTTATTC	ATAGGCTTTT	1800
CCCAGATGCA	ATTACCATTG	GTGAAGATGT	TAGCGGAATG	CCGACATTTT	GTATTCCCGT	1860
TCAAGATGGG	GGTGTGGCT	TTGACTATCG	GCTGCATATG	GCAATTGCTG	ATAAATGGAT	1920
TGAGTTGCTC	AAGAAACGGG	ATGAGGATTG	GAGAGTGGGT	GATATTGTTC	ATACACTGAC	1980
AAATAGAAGA	TGGTCGGAAA	AGTGTGTTTC	ATACGCTGAA	AGTCATGATC	AAGCTCTAGT	2040
CGGTGATAAA	ACTATAGCAT	TCTGGCTGAT	GGACAAGGAT	ATGTATGATT	TTATGGCTCT	2100
GGATAGACCG	CCAACATCAT	TAATAGATCG	TGGGATAGCA	TTGCACAAGA	TGATTAGGCT	2160
TGTAACATATG	GGATTAGGAG	GAGAAGGGTA	CCTAAATTTT	ATGGGAAATG	AATTCGGCCA	2220
CCCTGAGTGG	ATTGATTTCC	CTAGGGCTGA	GCCACACCTT	TCTGATGGCT	CAGTAATTCC	2280
CGGAAACCAA	TTCAGTTATG	ATAAATGCAG	ACGGAGATTT	GACCTGGGAG	ATGCAGAATA	2340
TTTAAGATAC	CATGGGTTAC	AAGAATTTGA	CTGGGCTATG	CAGTATCTTG	AAGATAAATA	2400
TGAGTTTATG	ACTTCAGAAC	ACCAGTTCAT	ATCACGAAAG	GATGAAGGAG	ATAGGATGAT	2460
TGTATTTGAA	AGAGGAAACC	TAGTTTTTCGT	CTTTAATTTT	CACTGGACAA	ATAGCTATTC	2520
AGACTATCGC	ATAGGCTGCC	TGAAGCCTGG	AAAATACAAG	GTTGTCTTGG	ACTCAGATGA	2580
TCCACTTTTT	GGTGGCTTCG	GGAGAATTGA	TCATAATGCC	GAATATTTCA	CCTCTGAAGG	2640
ATCGTATGAT	GATCGTCCTT	GTTCAATTAT	GGTGTATGCA	CCTAGTAGAA	CAGCAGTGGT	2700
CTATGCACTA	GTAGACAAAC	TAGAAGTAGC	AGTAGTAGAA	GAACCCATTG	AAGAATGAAC	2760
GAAC TTGTGA	TCGCGTTGAA	AGATTTGAAC	GTTACTTGGT	CATCCACATA	GAGCTTCTTG	2820
ACATCAGTCT	TGGCGGAATT	GCATGTGACA	ACAAGGTTTG	CAGTTCTTTC	CACTATTAGT	2880
AGTCCACCGA	TATACGCAGA	GATGAAGTGC	TGAACAAACA	TATGTAAAAT	CGATGAATTT	2940
ATGTCGAATG	CTGGGACGAT	CGAATTCCTG	CAGCC			2975

CTA Leu	CAA Gln	CTA Leu 140	CAA Gln	GAA Glu	GGT Gly	GGT Gly	AAA Lys 145	CTG Leu	GAG Glu	GAG Glu	TCT Ser	AAA Lys 150	ACA Thr	TTA Leu	AAT Asn	603
ACT Thr	TCT Ser 155	GAA Glu	GAG Glu	ACA Thr	ATT Ile	ATT Ile 160	GAT Asp	GAA Glu	TCT Ser	GAT Asp	AGG Arg 165	ATC Ile	AGA Arg	GAG Glu	AGG Arg	651
GGC Gly 170	ATC Ile	CCT Pro	CCA Pro	CCT Pro	GGA Gly 175	CTT Leu	GGT Gly	CAG Gln	AAG Lys	ATT Ile 180	TAT Tyr	GAA Glu	ATA Ile	GAC Asp	CCC Pro 185	699
CTT Leu	TTG Leu	ACA Thr	AAC Asn	TAT Tyr 190	CGT Arg	CAA Gln	CAC His	CTT Leu	GAT Asp 195	TAC Tyr	AGG Arg	TAT Tyr	TCA Ser	CAG Gln 200	TAC Tyr	747
AAG Lys	AAA Lys	CTG Leu	AGG Arg 205	GAG Glu	GCA Ala	ATT Ile	GAC Asp	AAG Lys 210	TAT Tyr	GAG Glu	GGT Gly	GGT Gly	TTG Leu 215	GAA Glu	GCC Ala	795
TTT Phe	TCT Ser	CGT Arg 220	GGT Gly	TAT Tyr	GAA Glu	AAA Lys	ATG Met 225	GGT Gly	TTC Phe	ACT Thr	CGT Arg	AGT Ser 230	GCT Ala	ACA Thr	GGT Gly	843
ATC Ile	ACT Thr 235	TAC Tyr	CGT Arg	GAG Glu	TGG Trp	GCT Ala 240	CTT Leu	GGT Gly	GCC Ala	CAG Gln	TCA Ser 245	GCT Ala	GCC Ala	CTC Leu	ATT Ile	891
GGA Gly 250	GAT Asp	TTC Phe	AAC Asn	AAT Asn	TGG Trp 255	GAC Asp	GCA Ala	AAT Asn	GCT Ala	GAC Asp 260	ATT Ile	ATG Met	ACT Thr	CGG Arg	AAT Asn 265	939
GAA Glu	TTT Phe	GGT Gly	GTC Val	TGG Trp 270	GAG Glu	ATT Ile	TTT Phe	CTG Leu	CCA Pro 275	AAT Asn	AAT Asn	GTG Val	GAT Asp	GGT Gly 280	TCT Ser	987
CCT Pro	GCA Ala	ATT Ile	CCT Pro 285	CAT His	GGG Gly	TCC Ser	AGA Arg	GTG Val 290	AAG Lys	ATA Ile	CGT Arg	ATG Met 295	GAC Asp	ACT Thr	CCA Pro	1035
TCA Ser	GGT Gly	GTT Val 300	AAG Lys	GAT Asp	TCC Ser	ATT Ile	CCT Pro 305	GCT Ala	TGG Trp	ATC Ile	AAC Asn	TAC Tyr 310	TCT Ser	TTA Leu	CAG Gln	1083
CTT Leu	CCT Pro 315	GAT Asp	GAA Glu	ATT Ile	CCA Pro	TAT Tyr 320	AAT Asn	GGA Gly	ATA Ile	CAT His	TAT Tyr 325	GAT Asp	CCA Pro	CCC Pro	GAA Glu	1131
GAG Glu 330	GAG Glu	AGG Arg	TAT Tyr	ATC Ile	TTC Phe 335	CAA Gln	CAC His	CCA Pro	CGG Arg	CCA Pro 340	AAG Lys	AAA Lys	CCA Pro	AAG Lys	TCG Ser 345	1179
CTG Leu	AGA Arg	ATA Ile	TAT Tyr	GAA Glu 350	TCT Ser	CAT His	ATT Ile	GGA Gly	ATG Met 355	AGT Ser	AGT Ser	CCG Pro	GAG Glu	CCT Pro 360	AAA Lys	1227

45

ATG GCA ATT GCT GAT AAA CGG ATT GAG TTG CTC AAG AAA CGG GAT GAG Met Ala Ile Ala Asp Lys Arg Ile Glu Leu Leu Lys Lys Arg Asp Glu 590 595 600	1947
GAT TGG AGA GTG GGT GAT ATT GTT CAT ACA CTG ACA AAT AGA AGA TGG Asp Trp Arg Val Gly Asp Ile Val His Thr Leu Thr Asn Arg Arg Trp 605 610 615	1995
TCG GAA AAG TGT GTT TCA TAC GCT GAA AGT CAT GAT CAA GCT CTA GTC Ser Glu Lys Cys Val Ser Tyr Ala Glu Ser His Asp Gln Ala Leu Val 620 625 630	2043
GGT GAT AAA ACT ATA GCA TTC TGG CTG ATG GAC AAG GAT ATG TAT GAT Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp Met Tyr Asp 635 640 645	2091
TTT ATG GCT CTG GAT AGA CCG TCA ACA TCA TTA ATA GAT CGT GGG ATA Phe Met Ala Leu Asp Arg Pro Ser Thr Ser Leu Ile Asp Arg Gly Ile 650 655 660 665	2139
GCA TTG CAC AAG ATG ATT AGG CTT GTA ACT ATG GGA TTA GGA GGA GAA Ala Leu His Lys Met Ile Arg Leu Val Thr Met Gly Leu Gly Gly Glu 670 675 680	2187
GGG TAC CTA AAT TTC ATG GGA AAT GAA TTC GGC CAC CCT GAG TGG ATT Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile 685 690 695	2235
GAT TTC CCT AGG GCT GAA CAA CAC CTC TCT GAT GGC TCA GTA ATC CCC Asp Phe Pro Arg Ala Glu Gln His Leu Ser Asp Gly Ser Val Ile Pro 700 705 710	2283
GGA AAC CAA TTC AGT TAT GAT AAA TGC AGA CGG AGA TTT GAC CTG GGA Gly Asn Gln Phe Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly 715 720 725	2331
GAT GCA GAA TAT TTA AGA TAC CGT GGG TTG CAA GAA TTT GAC CGG CCT Asp Ala Glu Tyr Leu Arg Tyr Arg Gly Leu Gln Glu Phe Asp Arg Pro 730 735 740 745	2379
ATG CAG TAT CTT GAA GAT AAA TAT GAG TTT ATG ACT TCA GAA CAC CAG Met Gln Tyr Leu Glu Asp Lys Tyr Glu Phe Met Thr Ser Glu His Gln 750 755 760	2427
TTC ATA TCA CGA AAG GAT GAA GGA GAT AGG ATG ATT GTA TTT GAA AAA Phe Ile Ser Arg Lys Asp Glu Gly Asp Arg Met Ile Val Phe Glu Lys 765 770 775	2475
GGA AAC CTA GTT TTT GTC TTT AAT TTT CAC TGG ACA AAA AGC TAT TCA Gly Asn Leu Val Phe Val Phe Asn Phe His Trp Thr Lys Ser Tyr Ser 780 785 790	2523
GAC TAT CGC ATA GCC TGC CTG AAG CCT GGA AAA TAC AAG GTT GCC TTG Asp Tyr Arg Ile Ala Cys Leu Lys Pro Gly Lys Tyr Lys Val Ala Leu 795 800 805	2571

Ala Ser Thr Asp Val Asp Ser Ser Thr Met Glu His Ala Ser Gln Ile
 100 105 110
 Lys Thr Glu Asn Asp Asp Val Glu Pro Ser Ser Asp Leu Thr Gly Ser
 115 120 125
 Val Glu Glu Leu Asp Phe Ala Ser Ser Leu Gln Leu Gln Glu Gly Gly
 130 135 140
 Lys Leu Glu Glu Ser Lys Thr Leu Asn Thr Ser Glu Glu Thr Ile Ile
 145 150 155 160
 Asp Glu Ser Asp Arg Ile Arg Glu Arg Gly Ile Pro Pro Pro Gly Leu
 165 170 175
 Gly Gln Lys Ile Tyr Glu Ile Asp Pro Leu Leu Thr Asn Tyr Arg Gln
 180 185 190
 His Leu Asp Tyr Arg Tyr Ser Gln Tyr Lys Lys Leu Arg Glu Ala Ile
 195 200 205
 Asp Lys Tyr Glu Gly Gly Leu Glu Ala Phe Ser Arg Gly Tyr Glu Lys
 210 215 220
 Met Gly Phe Thr Arg Ser Ala Thr Gly Ile Thr Tyr Arg Glu Trp Ala
 225 230 235 240
 Leu Gly Ala Gln Ser Ala Ala Leu Ile Gly Asp Phe Asn Asn Trp Asp
 245 250 255
 Ala Asn Ala Asp Ile Met Thr Arg Asn Glu Phe Gly Val Trp Glu Ile
 260 265 270
 Phe Leu Pro Asn Asn Val Asp Gly Ser Pro Ala Ile Pro His Gly Ser
 275 280 285
 Arg Val Lys Ile Arg Met Asp Thr Pro Ser Gly Val Lys Asp Ser Ile
 290 295 300
 Pro Ala Trp Ile Asn Tyr Ser Leu Gln Leu Pro Asp Glu Ile Pro Tyr
 305 310 315 320
 Asn Gly Ile His Tyr Asp Pro Pro Glu Glu Glu Arg Tyr Ile Phe Gln
 325 330 335
 His Pro Arg Pro Lys Lys Pro Lys Ser Leu Arg Ile Tyr Glu Ser His
 340 345 350
 Ile Gly Met Ser Ser Pro Glu Pro Lys Ile Asn Ser Tyr Val Asn Phe
 355 360 365
 Arg Asp Glu Val Leu Pro Arg Ile Lys Lys Leu Gly Tyr Asn Ala Leu
 370 375 380
 Gln Ile Met Ala Ile Gln Glu His Ser Tyr Tyr Ala Ser Phe Gly Tyr
 385 390 395 400

His Leu Ser Asp Gly Ser Val Ile Pro Gly Asn Gln Phe Ser Tyr Asp
 705 710 715 720
 Lys Cys Arg Arg Arg Phe Asp Leu Gly Asp Ala Glu Tyr Leu Arg Tyr
 725 730 735
 Arg Gly Leu Gln Glu Phe Asp Arg Pro Met Gln Tyr Leu Glu Asp Lys
 740 745 750
 Tyr Glu Phe Met Thr Ser Glu His Gln Phe Ile Ser Arg Lys Asp Glu
 755 760 765
 Gly Asp Arg Met Ile Val Phe Glu Lys Gly Asn Leu Val Phe Val Phe
 770 775 780
 Asn Phe His Trp Thr Lys Ser Tyr Ser Asp Tyr Arg Ile Ala Cys Leu
 785 790 795 800
 Lys Pro Gly Lys Tyr Lys Val Ala Leu Asp Ser Asp Asp Pro Leu Phe
 805 810 815
 Gly Gly Phe Gly Arg Ile Asp His Asn Ala Glu Tyr Phe Thr Phe Glu
 820 825 830
 Gly Trp Tyr Asp Asp Arg Pro Arg Ser Ile Met Val Tyr Ala Pro Cys
 835 840 845
 Lys Thr Ala Val Val Tyr Ala Leu Val Asp Lys Glu Glu Glu Glu Glu
 850 855 860
 Glu Glu Glu Glu Glu Glu Val Ala Ala Val Glu Glu Val Val Val Glu
 865 870 875 880
 Glu Glu

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2576 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TCATTAAAGA GGAGAAATTA ACTATGAGAG GATCTCACCA TCACCATCAC CATGGGATCT	60
TGGCTGAAAA GTCTTCTTAC AATTCCGAAT TCCGACCTTC TACAGTTGCA GCATCGGGGA	120
AAGTCCTTGT GCCTGGAACC CAGAGTGATA GCTCCTCATC CTCAACAAAC CAATTTGAGT	180
TCACTGAGAC ATCTCCAGAA AATTCCCCAG CATCAACTGA TG TAGATAGT TCAACAATGG	240
AACACGCTAG CCAGATTAAA ACTGAGAACG ATGACGTTGA GCCGTCAAGT GATCTTACAG	300

GGAAATGAAT TCGGCCACCC TGAGTGGATT GATTTCCTTA GGGCTGAACA ACACCTCTCT	2040
GATGACTCAG TAATTCCCGG AAACCAATTC AGTTATGATA AATGCAGACG GAGATTTGAC	2100
CTGGGAGATG CAGAATATTT AAGATACCGT GGGTTGCAAG AATTTGACCG GGCTATGCAG	2160
TATCTTGAAG ATAAATATGA GTTTATGACT TCAGAACACC AGTTCATATC ACGAAAGGAT	2220
GAAGGAGATA GGATGATTGT ATTTGAAAAA GGAAACCTAG TTTTGTCTT TAATTTTCAC	2280
TGGACAAAAA GCTATTCAGA CTATCGCATA GGCTGCCTGA AGCCTGGAAA ATACAAGGTT	2340
GCCTTGGACT CAGATGATCC ACTTTTTGGT GGCTTCGGGA GAATTGATCA TAATGCCGAA	2400
TATTTACCT TTGAAGGATG GTATGATGAT CGTCCTCGTT CAATTATGGT GTATGCACCT	2460
TGTAGAACAG CAGTGGTCTA TGCACTAGTA GACAAAGAAG AAGAAGAAGA AGAAGAAGAA	2520
GAAGAAGTAG CAGTAGTAGA AGAAGTAGTA GTAGAAGAAG AATGAACGAA CTTGTG	2576

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2529 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGATGCTAAT GTTCTGTAT TCTTGAAAAA GCACTCTCTT TCACGGAAGA TCTTGGCTGA	60
AAAGTCTTCT TACAATTCCG AATCCCGACC TTCTACAGTT GCAGCATCGG GGAAAGTCCT	120
TGTGCCTGGA AYCCAGAGTG ATAGCTCCTC ATCCTCAACA GACCAATTTG AGTTCCTGA	180
GACATCTCCA GAAAATTCCC CAGCATCAAC TGATGTAGAT AGTTCAACAA TGGAACACGC	240
TAGCCAGATT AAAACTGAGA ACGATGACGT TGAGCCGTCA AGTGATCTTA CAGGAAGTGT	300
TGAAGAGCTG GATTTTGCTT CATCACTACA ACTACAAGAA GGTGGTAAAC TGGAGGAGTC	360
TAAACATTA AATACTTCTG AAGAGACAAT TATTGATGAA TCTGATAGGA TCAGAGAGAG	420
GGGCATCCCT CCACCTGGAC TTGGTCAGAA GATTTATGAA ATAGACCCCC TTTTGACAAA	480
CTATCGTCAA CACCTTGATT ACAGGTATTC ACAGTACAAG AACTGAGGG AGGCAATTGA	540
CAAGTATGAG GGTGGTTTGG AAGCTTTTTC TCGTGGTTAT GAAAAAATGG GTTTCACCTG	600
TAGTGCTACA GGTATCACTT ACCGTGAGTG GGCTCCTGGT GCCCAGTCAG CTGCCCTCAT	660
TGGAGATTTT AACAATTGGG ACGCAAATGC TGACATTATG ACTCGGAATG AATTTGGTGT	720
CTGGGAGATT TTTCTGCCAA ATAATGTGGA TGGTTCTCCT GCAATTCCTC ATGGGTCCAG	780

AACAGCAGTG GTCTATGCAC TAGTAGACAA ANTAGAAGNA GAAGAAGAAG AAGAANCCGN 2520
NGAAGAATT 2529

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3231 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GATTTAATAC GACTCACTAT AGGGATTTTT TTTTTTTTTT TTTTAAAAAC CTCCTCCACT 60
CAGTCTTGGG ATCTCTCTCT CTCTTCACGC TTCTCTTGGG GCCTTGAAC T CAGCAATTTG 120
ACACTCAGTT AGTTACACTC CTATCACTCA TCAGATCTCT ATTTTTTCTC TTAATTCCAA 180
CCAAGGAATG AATTAAGA TTAGATTTGA AGGAGAGAAG AAGAAAGATG GTGTATACAC 240
TCTCTGGAGT TCGTTTTCTT ACTGTTCCAT CAGTGTACAA ATCTAATGGA TTCAGCAGTA 300
ATGGTGATCG GAGGAATGCT AATGTTTCTG TATTCTTGAA AAAGCACTCT CTTTCACGGA 360
AGATCTTGGC TGAAAAGTCT TCTTACGATT CCGAATCCCG ACCTTCTACA GTTGCAGCAT 420
CGGGGAAAGT CTTGTACCT GGAATCCAGA GTGATAGCTC CTCATCCTCA ACAGACCAAT 480
TTGAGTTCAC TGAGACAGCT CCAGAAAATT CCCCAGCATC AACTGATGTG GATAGTTCAA 540
CAATGGAACA CGCTAGCCAG ATTAATACTG AGAACGATGA CGTTGAGCCG TCAAGTGATC 600
TTACAGGAAG TGTTGAAGAG TTGGATTTTG CTTCACTACT ACAACTACAA GAAGGTGGTA 660
AACTGGAGGA GTCTAAAACA TTAAATACTT CTGAAGAGAC AATTATTGAT GAATCTGATA 720
GGATCAGAGA GAGGGGCATC CCTCCACCTG GACTTGGTCA GAAGATTTAT GAAATAGACC 780
CCCTTTTGAC AACTATCGT CAACACCTTG ATTACAGGTA TTCACAGTAC AAGAAAATGA 840
GGGAGGCAAT TGACAAGTAT GAGGGTGGTT TGAAGCTTT TTCTCGTGGT TATGAAAAAA 900
TGGGTTTCAC TCGTAGTGCT ACAGGTATCA CTTACCGTGA GTGGGCTCCT GGTGCCCAGT 960
CAGCTGCTCT CATTGGAGAT TTCAACAATT GGGACGCAAA TGCTGACATT ATGACTCGGA 1020
ATGAATTTGG TGTCTGGGAG ATTTTCTGC CAAATAATGT GGATGGTTCT CCTGCAATTC 1080
CTCATGGGTC CAGAGTGAAG ATACGCATGG ACACTTCATC AGGTGTTAAG GATTCCATTC 1140
CTGCTTGGAT CAACTACTCT TTACAGCTTC CTGATGAAAT TCCATATAAT GGAATATATT 1200
ATGATCCACC CGAAGAGGAG AGGTATGTCT TCCAACACCC ACGGCCAAAG AAACCAAAGT 1260

GCGGAATTTT	ATGTGACAAA	AGGTTTGCAA	TTCTTTCCAC	TATTAGTAGT	GCAACGATAT	3000
ACGCAGAGAT	GAAGTGCTGA	ACAAACATAT	GTAAAATCGA	TGAATTTATG	TCGAATGCTG	3060
GGACGGGCTT	CAGCAGGTTT	TGCTTAGTGA	GTTCTGTAAA	TTGTCATCTC	TTTANATGTA	3120
CAGCCCACTA	GAAATCAATT	ATGTGAGACC	TAAAAAACAA	TAACCATAAA	ATGGAAATAG	3180
TGCTGATCTA	ATGATGTTTT	AANCCNNNNA	AAAAAAAAAA	AAAAACTCGA	G	3231

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2578 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TCATTAAAGA	GGAGAAATTA	ACTATGAGAG	GATCTCACCA	TCACCATCAC	CATGGGATCT	60
TGGCTGAAAA	GTCTTCTTAC	AATTCCGAAT	TCCGACCTTC	TACAGTTGCA	GCATCGGGGA	120
AAGTCCTTGT	GCCTGGAACC	CAGAGTGATA	GCTCCTCATC	CTCAACAAAC	CAATTTGAGT	180
TCACTGAGAC	ATCTCCAGAA	AATTCCTCAG	CATCAACTGA	TGTAGATAGT	TCAACAATGG	240
AACACGCTAG	CCAGATTAAA	ACTGAGAACG	ATGACGTTGA	GCCGTCAAGT	GATCTTACAG	300
GAAGTGTTGA	AGAGCTGGAT	TTTGCTTCAT	CACTACAAC	ACAAGAAGGT	GGTAAACTGG	360
AGGAGTCTAA	AACATTAAAT	ACTTCTGAAG	AGACAATTAT	TGATGAATCT	GATAGGATCA	420
GAGAGAGGGG	CATCCCTCCA	CCTGGACTTG	GTCAGAAGAT	TTATGAAATA	GACCCCTTTT	480
TGACAAACTA	TCGTCAACAC	CTTGATTACA	GGTATTCACA	GTACAAGAAA	CTGAGGGAGG	540
CAATTGACAA	GTATGAGGGT	GGTTTGGAAG	CTTTTCTCG	TGGTTATGAA	AAAATGGGTT	600
TCACTCGTAG	TGCTACAGGT	ATCACTTACC	GTGAGTGGGC	TCCTGGTGCC	CAGTCAGCTG	660
CCCTCATTGG	AGATTTCAAC	AATTGGGACG	CAAATGCTGA	CATTATGACT	CGGAATGAAT	720
TTGGTGTCTG	GGAGATTTTT	CTGCCAAATA	ATGTGGATGG	TTCTCCTGCA	ATTCCTCATG	780
GGTCCAGAGT	GAAGATACGT	ATGGACACTC	CATCAGGTGT	TAAGGATTCC	ATTCCTGCTT	840
GGATCAACTA	CTCTTCACAG	CTTCCTGATG	AAATTCCATA	TAATGGAATA	TATTATGATC	900
CACCCGAAGA	GGAGAGGTAT	ATCTTCCAAC	ACCCACGGCC	AAAGAAACCA	AAGTCGCTGA	960
GAATATATGA	ATCTCATATT	GGAATGAGTA	GTCCGGAGCC	TAAAATTAAC	TCATACGTGA	1020
ATTTTAGAGA	TGAAGTTCTT	CCTCGCATAA	AAAAGCTTGG	GTACAATGCG	GTGCAAATTA	1080

57

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

AATTTYATGG GNAAYGARTT YGG

23

9. Starch which as extracted from a potato plant by wet milling at ambient temperature has a pasting viscosity in the range 214 - 434 SNU's, as judged by viscoamylograph conducted according to the protocol defined in claim 7.
10. Starch which as extracted from a potato plant by wet milling at ambient temperature has a set-back viscosity in the range 450 - 618 SNU's, as judged by viscoamylograph conducted according to the protocol defined in claim 7.
11. Starch which as extracted from a potato plant by wet milling at ambient temperature has a set-back viscosity in the range 14 - 192 SNU's, as judged by viscoamylograph conducted according to the protocol defined in claim 7.
12. Starch which as extracted from a potato plant by wet milling at ambient temperature has a peak viscosity in the range 200 - 500 SNU's and a set-back viscosity in the range 275-618 SNU's as judged by viscoamylograph according to the protocol defined in claim 7.
13. Starch which as extracted from a potato plant by wet milling at ambient temperature has a viscosity which does not decrease between the start of the heating phase (step 2) and the start of the final holding phase (step 5) and has a set-back viscosity of 303 SNU's or less as judged by viscoamylograph according to the protocol defined in claim 7.
14. Starch which as extracted from a potato plant by wet milling at ambient temperature displays no significant increase in viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7.
15. Starch which as extracted from a potato plant by wet milling at ambient temperature, is in accordance with claim 7 and in accordance with any one of claims 8 to 14.
16. Starch according to any one of claims 7 to 15, having an amylose content in the range 35 - 66%, as judged by the method of Morrison & Laignelet defined in claim 1.

enzyme (SBE) obtainable from potato plants.

28. A nucleotide sequence according to claim 27, encoding a polypeptide comprising substantially the amino acid sequence of residues 49 to 882 of the sequence shown in Figure 5.

29. A nucleotide sequence according to claim 27 or 28, comprising substantially the sequence of nucleotides 289 to 2790 of the sequence shown in Figure 5, or a functional equivalent thereof.

30. A nucleotide sequence according to claim 29, further comprising the sequence of nucleotides 145 to 288 of the sequence shown in Figure 5, or a functional equivalent thereof.

31. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 228 to 2855 of the sequence labelled psbe2con.seq in Figure 8, or a functional equivalent thereof.

32. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 57 to 2564 of the sequence labelled as psbe2con.seq in Figure 12, or a functional equivalent thereof.

33. A nucleotide sequence according to any one of claims 27 to 32, comprising an in-frame ATG start codon, and optionally including a 5' and/or a 3' untranslated region.

34. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 45 to 3200 of the sequence labelled as psbe2con.seq in Figure 8, or a functional equivalent thereof.

35. A nucleic acid construct comprising a sequence in accordance with any one of claims 27 to 34.

introducing into the plant one or more further sequences.

46. A method according to claim 45, wherein one or more of the further sequences are operably linked in the anti-sense orientation to a suitable promoter active in the plant.

47. A method according to claim 45 or 46, wherein the further sequence comprises a portion of a class B SBE nucleotide sequence.

48. A method according to any one of claims 42 to 47, effective in altering the starch composition of a plant.

49. A plant or plant cell having characteristics altered by the method of any one of claims 42 to 48, or the progeny of such a plant, or part of such a plant.

50. A plant according to claim 49, selected from one of the following: potato, pea, tomato, maize, wheat, rice, barley, sweet potato, and cassava.

51. A tuber or other storage organ from a plant according to claim 49 or 50.

52. Use of a tuber or other storage organ according to claim 51, in the preparation and/or processing of a foodstuff.

53. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an elevated viscosity onset temperature as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

54. A plant according to claim 53, wherein the viscosity onset temperature is elevated by an amount in the range of 10 to 25°C.

55. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has a decreased peak viscosity as judged by

63. A plant according to claim 49 or 50, containing starch which, as extracted from the plant, has a phosphorus content in excess of 200mg/100grams dry weight starch.
64. Starch obtainable from a plant according to any one of claims 49, 50 or 53 - 63.
65. Starch according to claim 64 and further in accordance with any one of claims 1 - 22.
66. A method of modifying starch *in vitro*, comprising treating starch under suitable conditions with an effective amount of a polypeptide in accordance with any one of claims 38 to 41.
67. A potato plant or part thereof which, in its wild type possesses an effective SBE A gene, but which plant has been altered such that there is no effective expression of an SBE A polypeptide within the cells of at least part of the plant.
68. A potato plant according to claim 67, wherein the alteration is effected by a method according to any one of claims 42-48.

2/75

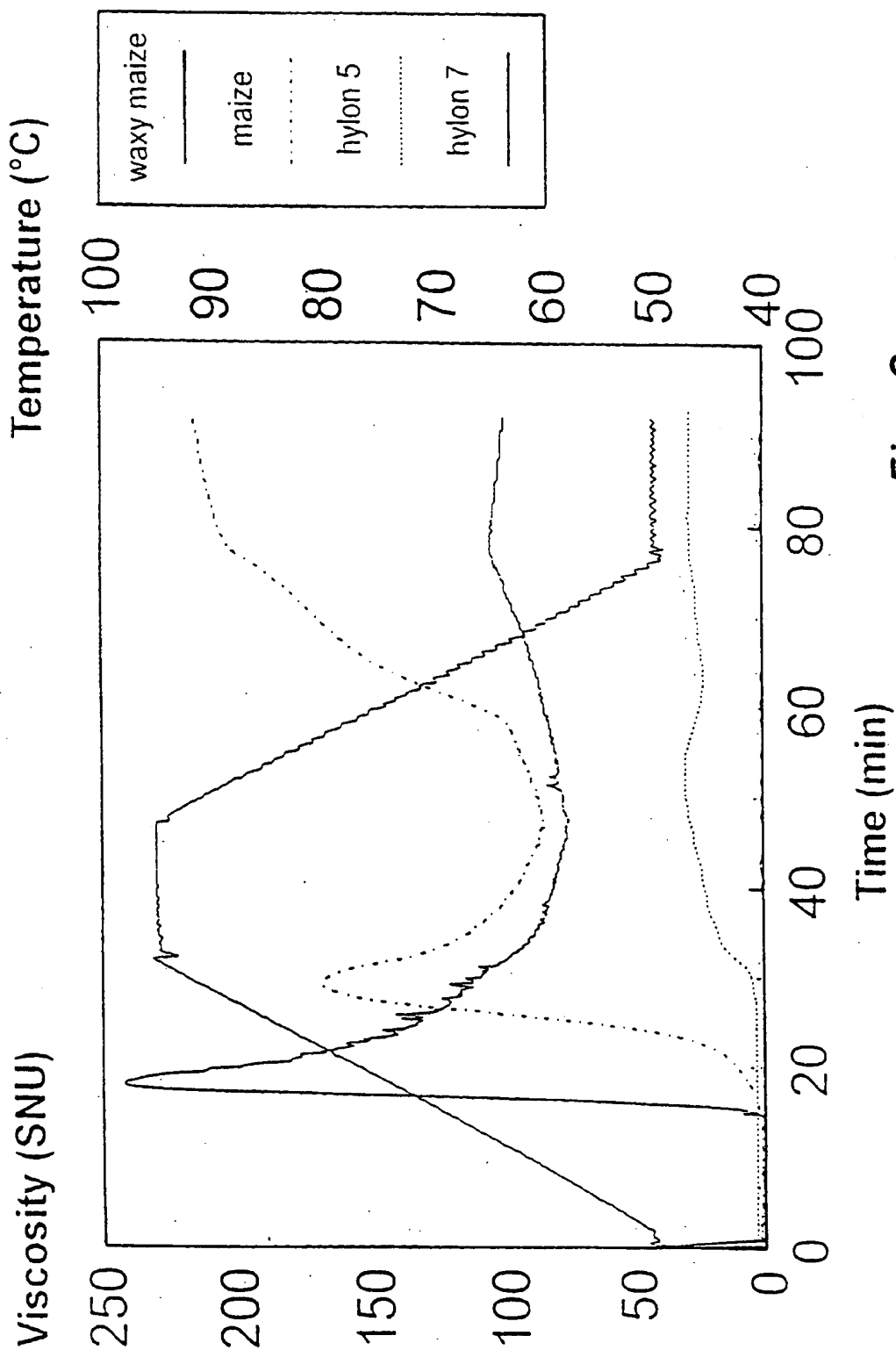


Fig. 2

4/75
Fig. 4a
Sheet 2

Majority P A S P T I D R G I A L H K M I H L I T M G L G G E G Y L N F M G N

maize 2

pea 1

maize 1

rice 1

potato 1

human

P S T P T I D R G I A L H K M I R L I T M G L G G E G Y L N F M G N
P S T P L I D R G I A L H K M I R L I T M G L G G E G Y L N F M G N
P A S P T I D R G I A L H K M I R L I T M G L G G E G Y L N F M G N
P A S P T I N D R G I A L H K M I R L I T M G L G G E G Y L N F M G N
D A S P V V D A G I A L H K M I R L I T M G L G G E G Y L N F M G N
P F T P V I D R G I A L H K M I R L I T M G L G G E G Y L N F M G N

Majority F S L G D A D H L R Y K G M N A F D Q A M N A L E E K F S F L A S S

maize 2

pea 1

maize 1

rice 1

potato 1

human

F D L G D A D Y L R Y H G M Q E F D Q A M Q H L E Q K Y E F M T S D
F D L G D A D Y L R Y H G M Q E F D Q A M Q H L E Q K Y E F M T S E
W S L V D T D H L R Y K Y M N A F F D Q A M Q H L E Q K Y E F M T S S
W S L V D T D H L R Y K Y M N A F F D Q A M Q H L E Q K Y E F M T S S
W N L A D S E H L R Y K Y M N A F F D Q A M Q H L E Q K Y E F M T S S
F H L T D D L L R Y K Y M N A F F D Q A M Q H L E Q K Y E F M T S S

Majority K V G C D L P G K Y K V A L D S D A L V F G G H G R V G H D V D H F

maize 2

pea 1

maize 1

rice 1

potato 1

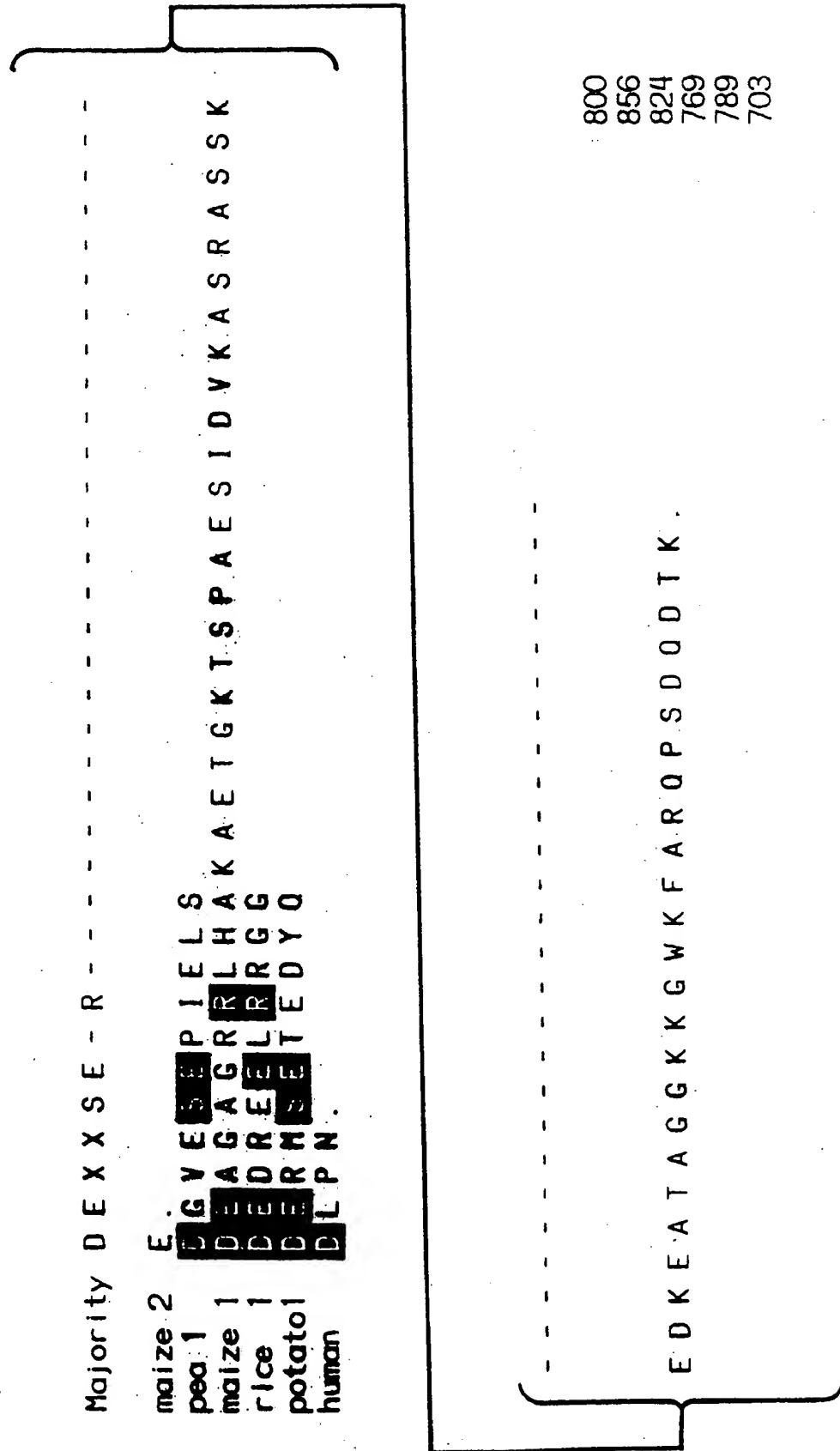
human

R I G C R K P G V Y K V V L D S D A G L F G G F S R I H H A A E H F
K V G C L K P G K Y K I V L D S D D T L F G G F N R R L N H T A E Y E F
K V G C D L P G K Y R V A L D S D A L V F G G H G R V G H D V D H F
K V G C D L P G K Y R V A L D S D A L V F G G H G R V G H D V D H F
K V G C D L P G K Y R V A L D S D A L V F G G H G R V G H D V D H F
R V G T A L P G K F K I V L D S D A A E Y G G H Q R R L D H S T D F F

Fig. 4a SHEET 1

SUBSTITUTE SHEET (RULE 26)

6/75



SUBSTITUTE SHEET (RULE 26)

Fig. 4a SHEET 3

8/75

TTGATGGGGCCTTGAAGCTCAGCAATTTGACACTCAGTTAGTTACA
AACTACCCCGGAAGCTTGAGTCGTTAACTGTGAGTCAATCAATGT
AAGGAATGAATAAAAGGATAGATTTGTAAAAACCCTAAGGAGAGA
TTCCTTACTTATTTTCCTATCTAAACATTTTGGGATTCTCTCT
M N K R I D L
GTTCCATCAGTGTACAAATCTAATGGATTTCAGCAGTAATGGTGAT
CAAGGTAGTCACATGTTTAGATTACCTAAGTCGTCATTACCACTA
V P S V Y K S N G F S S N G D
Bgl II EcoR I
TCACGGAAGATCTTGGCTGAAAAGTCTTCTTACAATTCCGAATTC
AGTGCCTTCTAGAACCGACTTTTCAGAAGAATGTTAAGGCTTAAG
S R K I L A E K S S Y N S E F
ACCCAGAGTGATAGCTCCTCATCCTCAACAGACCAATTTGAGTTC
TGGGTCTCACTATCGAGGAGTAGGAGTTGTCTGGTTAACTCAAG
T Q S D S S S S S T D Q F E F
AGTTCAACAATGGAACACGCTAGCCAGATTAAAGTGAAGACGAT
TCAAGTTGTTACCTTGTGCGATCGGTCTAATTTTGACTCTTGCTA
S S T M E H A S Q I K T E N D
GATTTTGCTTCATCACTACAAGTACAAGAAGGTGGTAACTGGAG
CTAAAACGAAGTAGTGATGTTGATGTTCTTCCACCATTTGACCTC
D F A S S L Q L Q E G G K L E

Fig 5
Sheet 2

Fig. 5 SHEET 1

SUBSTITUTE SHEET (RULE 26)

10/75

TCTGATAGGATCAGAGAGAGGGGCATCCCTCCACCTGGACTTGGT
AGACTATCCTAGTCTCTCTCCCCGTAGGGAGGTGGACCTGAACCA
S D R I R E R G I P P P G L G
CACCTTGATTACAGGTATTCACAGTACAAGAACTGAGGGAGGCA
GTGGAACATAATGTCCATAAGTGTCATGTTCTTTGACTCCCTCCGT
H L D Y R Y S Q Y K K L R E A
GAAAAAATGGGTTTCACTCGTAGTGCTACAGGTATCACTTACCGT
CTTTTTTACCCAAAGTGAGCATCACGATGTCCATAGTGAATGGCA
E K M G F T R S A T G I T Y R
ACAATTGGGACGCAAATGCTGACATTATGACTCGGAATGAATTT
TTGTTAACCCTGCGTTTACGACTGTAATACTGAGCCTTACTTAAA
N N W D A N A D I M T R N E F
GCAATTCCTCATGGGTCCAGAGTGAAGATACGTATGGACACTCCA
CGTTAAGGAGTACCCAGGTCTCACTTCTATGCATACCTGTGAGGT
A I P H G S R V K I R M D T P

Fig. 5
Sheet 4

Fig. 5 SHEET 3

SUBSTITUTE SHEET (RULE 26)

12/75

CAGCTTCCTGATGAAATTCCATATAATGGAATACATTATGATCCA
GTCGAAGGAGTAGTTTAAGGTATATTACCTTATGTAATAC TAGGT
Q L P D E I P Y N G I H Y D P

CCAAAGTCGCTGAGAATATATGAATCTCATATTGGAATGAGTAGT
GGTTTCAGCGACTCTTATATACTTAGAGTATAACCTTACTCATCA
P K S L R I Y E S H I G M S S

Hind III

CTTCCTCGCATAAAAAAGCTTGGGTACAATGCGCTGCAAATTATG
GAAGGAGCGTATTTTTTTCGAACCCATGTTACGCGACGTTTAATAC
L P R I K K L G Y N A L Q I M

ACAAATTTTTTTTGCACCAAGCAGCCGTTTTTGAACGCCCGACGAC
TGTTTAAAAAAACGTGGTTCGTGGCAAAACCTTGCGGGCTGCTG
T N F F A P S S R F G T P D D

CTCATGGACATTGTTTCACAGCCATGCATCAAATAATACTTTAGAT
GAGTACCTGTAACAAGTGTCGGTACGTAGTTTATTATGAAATCTA
L M D I V H S H A S N N T L D

Fig.5
Sheet
6

Fig. 5 SHEET 5

SUBSTITUTE SHEET (RULE 26)

14/75

Sac I

GGAGCTCGTGGTTATCATTGGATGTGGGATTCCCGCCTCTTTAAC

CCTCGAGCACCAATAGTAACCTACACCCTAAGGGCGGAGAAATTG

G A R G Y H W M W D S R L F N

TGGTGGTTGGATGCGTTCAAATTTGATGGATTTAGATTTGATGGT

ACCACCAACCTACGCAAGTTTAAACTACCTAAATCTAAACTACCA

W W L D A F K F D G F R F D G

ACTGGGAACCTACGAGGAATACTTTGGACTCGCAACTGATGTGGAT

TGACCCTTGATGCTCCTTATGAAACCTGAGCGTTGACTACACCTA

T G N Y E E Y F G L A T D V D

TTCCCAGATGCAATTACCATTGGTGAAGATGTTAGCGGAATGCCG

AAGGGTCTACGTTAATGGTAACCACTTCTACAATCGCCTTACGGC

F P D A I T I G E D V S G M P

CGGCTGCATATGGCAATTGCTGATAAACGGATTGAGTTGCTCAAG

GCCGACGTATACCGTTAACGACTATTTGCCTAACTCAACGAGTTC

R L H M A I A D K R I E L L K

ACAAATAGAAGATGGTCGGAAAAGTGTGTTTCATACGCTGAAAGT

TGTTTATCTTCTACCAGCCTTTTCACACAAAGTATGCGACTTTCA

T N R R W S E K C V S Y A E S

Fig 5
Sheet 8

Fig. 5 SHEET 7

SUBSTITUTE SHEET (RULE 26)

16/75

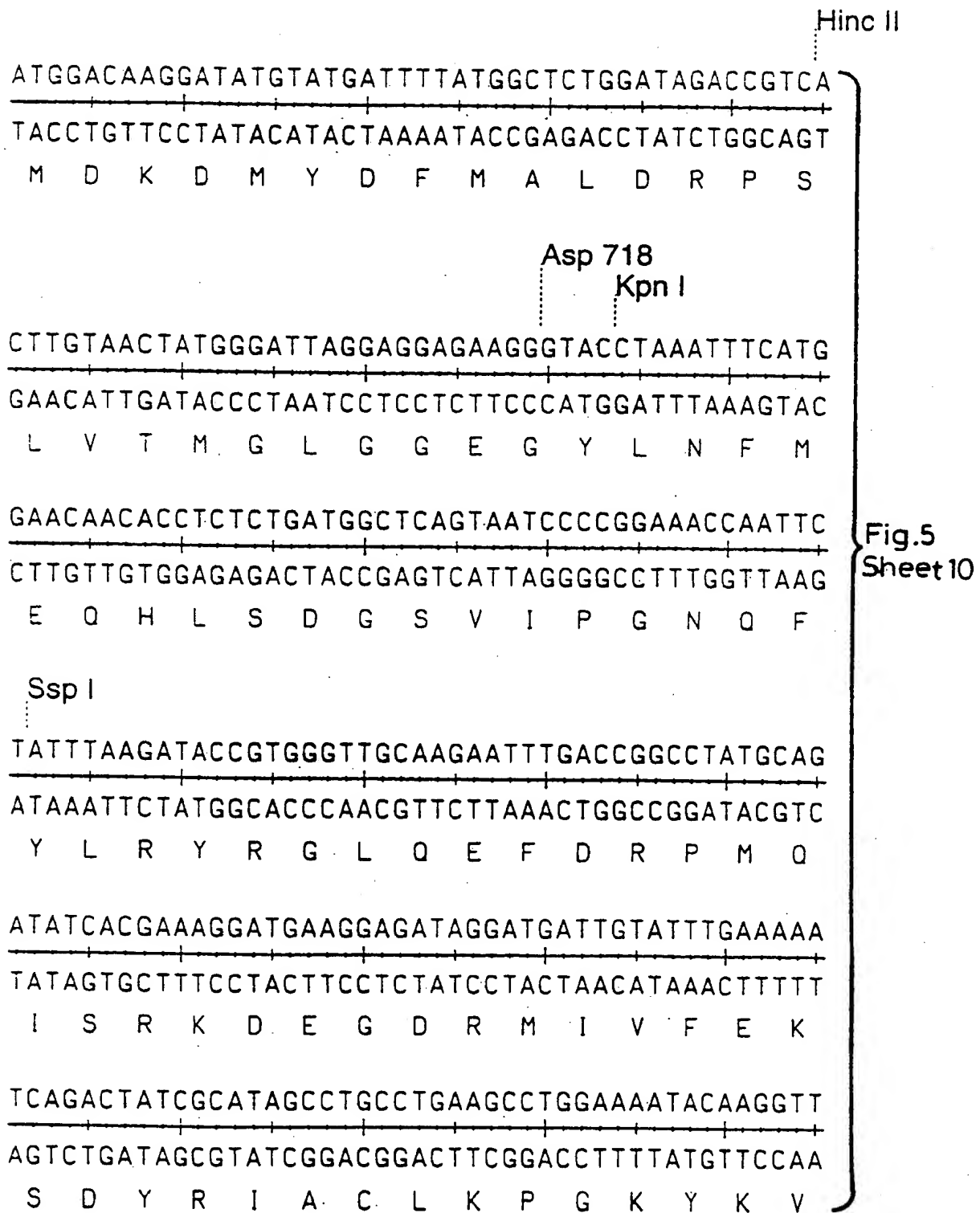


Fig.5 SHEET 9

SUBSTITUTE SHEET (RULE 26)

18/75

Ssp I

GATCATAATGCCGAATATTTACCTTTGAAGGATGGTATGATGAT

CTAGTATTACGGCTTATAAAGTGGAACTTCCTACCATACTACTA

D H N A E Y F T F E G W Y D D

GTCTATGCACTAGTAGACAAAGAAGAAGAAGAAGAAGAAGAA

CAGATACGTGATCATCTGTTTCTTCTTCTTCTTCTTCTTCTT

V Y A L V D K E E E E E E E E

TGAACGAACTTGTGATCGCGTTGAAAGATTTGAACGCTACATAGA

ACTTGCTTGAACACTAGCGCAACTTTCTAAACTTGCGATGTATCT

TCATGTGACACAAGGTTTGCAATTCTTTCCACTATTAGTAGTGCA

AGTACACTGTGTTCCAAACGTTAAGAAAGGTGATAATCATCACGT

EcoR I

Pst I

GATGAATTTATGTGAATGCTGGGACGATCGAATTCCTGCAGGCC

CTACTTAAATACAGCTTACGACCCTGCTAGCTTAAGGACGTCCGG

Fig 5
Sheet
12

Fig. 5 SHEET 11

SUBSTITUTE SHEET (RULE 26)

20/75

↘180 ↘190 ↘200 ↘210 ↘220
 IYEIDPLL TN YRQHL DY RYSQYKKL REAID KYEGGLEAF SRGYEKM GFTR
 : : DP L. Y : H: . R : : Y : : I: KYEG LE. F: : GY K. GF. R
 LLNL DPTLEPYLDHFRHRMKRYVDQKMLIEKYEGPLEEFAQGYLKFGFN R
 ↗100 ↗110 ↗120 ↗130 ↗140
 ↘230 ↘240 ↘250 ↘260 ↘270
 SATGITYREWALGAQSAALIGDFNNWDANADIMTRNEFGVWEIFLPNNVD
 ... I. YREWA : AQ. A. : IGDFN. W: : : : M. : : : FGVW. I : P: VD
 EDGCIVYREWAPAAQEA EVIGDFNGWNGSNHMM EK DQFGVWSIRIPD-VD
 ↗150 ↗160 ↗170 ↗180 ↗190
 ↘280 ↘290 ↘300 ↘310 ↘320
 GSPAIPHGSRVKIRMDTPSGV-KDSIPAWINYSLQLPDEI--PYNGIHYD
 : . P. IPH. SRVK: R. : GV D. IPAWI: Y: : : : PY: G: . D
 SKPVIPHNSRVKFRFKHGNVWVDRIPAWIKYATADATKFAAPYDGVYWD
 ↗200 ↗210 ↗220 ↗230 ↗240
 ↘330 ↘340 ↘350 ↘360 ↘370
 PPEEERYIFQHPRPKPKSLRIYESHIGMSSPEPKINSYVNFRDEVLPRI
 PP . ERY F: . PRP KP: : RIYE: H: GMSS: EP: : NSY : F D: VLPRI
 PPPSERYHFKYPRPPKPRAPRIYEAHVGMSSSEPRVNSYREFADDVLPRI
 ↗250 ↗260 ↗270 ↗280 ↗290
 ↘380 ↘390 ↘400 ↘410 ↘420
 KKLGYNALQIMAIQEHSYYASF GYHVTNFFAPSSRFGTPDDLKSLIDKAH
 K . YN: : Q: MAI EHSYY: SFGYHVTNFFA S: R: G. P: DLK LIDKAH
 KANNYNTVOLMAIMEHSYYGSF GYHVTNFFAVSNRYGNPEDLKYLIDKAH
 ↗300 ↗310 ↗320 ↗330 ↗340
 ↘430 ↘440 ↘450 ↘460 ↘470
 ELGIVVLMDIVHSHASNNTLDGLNMFDC---TDSCYFHSGARGYHWMWDS
 . LG: VL: D: VHSHASN. DGLN FD : : : YFH: G. RGYH : WDS
 SLGLQVLVDVVHSHASNNTDGLNGFDIGQGSQESYFHAGERGYHKLWDS
 ↗350 ↗360 ↗370 ↗380 ↗390
 ↘480 ↘490 ↘500 ↘510 ↘520
 RLFNYGNWEVLR YLLSNARWWLDAFKFDGFRFDGVTSM MYIHHGLSVGFT
 RLFNY: NWEVLR: LLSN RWWL: : : FDGFRFDG: TSM: Y: HHG: : : GFT
 RLFNYANWEVLRFLLSNLRWWLE EYNFDGFRFDGITSMLYVHHGINMGFT
 ↗400 ↗410 ↗420 ↗430 ↗440
 ↘530 ↘540 ↘550 ↘560 ↘570
 GNYEEYFGLATD VDAVVYLMLVNDLIHGLFPDAITIGEDVSGMPTFCIPV
 GNY: EYF: ATD VDAVVYLML. N: LIH : FPDA. . I: EDVSGMP. : . PV
 GNYNEYFSEATD VDAVVYLMLANL I HKIFPDATVIAEDVSGMPGLSRPV
 ↗450 ↗460 ↗470 ↗480 ↗490
 ↘580 ↘590 ↘600 ↘610 ↘620
 OEGGVGFDYRLHMAIADKRIELK-KRDEDWRVGDIVHTLTNRRWSEKCV
 EGG: GFDYRL MAI: DK: I: LK K. DEDW. : : : LTNRR: EKC:
 SEGGIGFDYRLAMAI PDKWIDY LKNKNDEDWSMKEVTSSLTNRRYTEKCI
 ↗500 ↗510 ↗520 ↗530 ↗540

Fig. 6 SHEET 1

SUBSTITUTE SHEET (RULE 26)

22/75

M V Y T L S G V R F P T V P S V Y K S N G F S S N G D R R N A N V S V F L K K H -- S L S R K I L A
 M V Y T : S G : R F P . : P S : . K S : . . D R R . : : S F L K : : S : S R . L
 M V Y T I S G I R F P V L P S L H K S --- T L R C D R R A S S H S F F L K N N S S S F S R T S L Y
 M V Y T I S G I R F P V L P S L H K S --- T L R C D R R A S S H S F F L K N N S S S F S R T S L Y
 E K S S Y N S E F R P S T V A A S G K V L V P G T Q S D S S S S S T D Q F E F T E T S P E N S P A S
 . K S : S E : : S T : A . S : K V L : P . . Q D : S S : D Q : E : E : . . .
 A K F S R D S E T K S S T I A E S D K V L I P E D Q - D N S V S L A D Q L E N P D I T S E D A Q N L
 T D V D S S T M E H A S Q I K T E N D D V E P S S D L T G S V E E L D F A S S L Q L O E G G K L E E
 . D : T M . : : : : . . . : . . . : . . . : . . . : . . . : S : : : : :
 E D L - - - T M K D G N K Y N I D - E S T S S Y R E V G D E K G S V T S S S L V D V N T D T Q - - A
 S K T L N T S E E T I I D E S D R I R E R G I P P P G L G Q K I Y E I D P L L T N Y R Q H L D Y R Y
 . K T S : . . . : . . : I I P P P G G Q K I Y E I D P L L . . R Q H L D : R Y
 K K T S V H S D K K V K V D K P K I - - - - I P P P G S G Q K I Y E I D P L L Q A H R Q H L D F R Y
 S Q Y K K L R E A I D K Y E G G L E A F S R G Y E K M G F T R S A T G I T Y R E W A L G A Q S A A L
 : Q Y K : : R E . I D K Y E G G L : A F S R G Y E K . G F T R S A T G I T Y R E W : G A : S A A L
 G O Y K R I R E E I D K Y E G G L D A F S R G Y E K F G F T R S A T G I T Y R E W G P G A K S A A L
 I G D F N N W D A N A D I M T R N E F G V W E I F L P N N V D G S P A I P H G S R V K I R M D T P S
 : G D F N N W : : N A D : M T : : . F G V W E I F L P N N . D G S P : I P H G S R V K I : M D T P S
 V G D F N N W N P N A D V M T K D A F G V W E I F L P N N A D G S P P I P H G S R V K I H M D T P S
 G V K D S I P A W I N Y S L Q L P D E I P Y N G I H Y D P P E E E R Y I F Q H P R P K K P K S L R I
 G : K D S I P A W I : : S : Q : P : E I P Y N G I . Y D P P E E E : Y : F : H P : P K : P : S : R I
 G I K D S I P A W I K F S V Q A P G E I P Y N G I Y Y D P P E E E K Y V F K H P Q P K R P Q S I R I
 Y E S H I G M S S P E P K I N S Y V N F R D E V L P R I K K L G Y N A L Q I M A I Q E H S Y Y A S F
 Y E S H I G M S S P E P K I N : Y . N F R D : V L P R I K K L G Y N A : Q I M A I Q E H S Y Y A S F
 Y E S H I G M S S P E P K I N T Y A N F R D D V L P R I K K L G Y N A V Q I M A I Q E H S Y Y A S F
 G Y H V T N F F A P S S R F G T P D D L K S L I D K A H E L G I V V L M D I V H S H A S N N T L D G
 G Y H V T N F F A P S S R F G T P : D L K S L I D : A H E L G : : V L M D I V H S H : S N N T L D G
 G Y H V T N F F A P S S R F G T P E D L K S L I D R A H E L G L L V L M D I V H S H S S N N T L D G

Fig. 7 SHEET 1

SUBSTITUTE SHEET (RULE 26)

24/75

1 -----TTG--AT-----
 1 -----TTGA-----
 1 -----GA-----
 45 **AAAAACGTCCTCCACTCAGTCTTGGCATCTCTGCTGCT**

 72 TTTCTCTTAATTCCAACCA**GGG**GAATGAATAAAAGGAT-A
 73 TTTCTCTTAATTCCAACCAAGG-AATGAATAAAAGGAT-A
 71 TTTCTCTTAATTCCAACCAAGG-AATGAATAAAAAGAT-A
 165 TTTCTCTTAATTCCAACCAAGG-AATGAAT**IAAA**AGAT**IA**

 191 TGTACAAATCTAATGGATT**CAGCAGTAATGGTGATCGGAG**
 191 TGTACAAATCTAATGGATT**CAGCAGTAATGGTGATCGGAG**
 189 TGTACAAATCTAATGGATT**CAGCAGTAATGGTGATCGGAG**
 274 TGTACAAATCTAATGGATT**CAGCAGTAATGGTGATCGGAG**

 311 AATTCCGACCTTCTACAGTTGCAGCATCGGGGAAAGTCCT
 311 AATTCCGACCTTCTACAGTTGCAGCATCGGGGAAAGTCCT
 309 AAT**CCG**ACCTTCTACA**A**TTGCAGCATCGGGGAAAGTCCT
 394 AAT**CCG**ACCTTCTACAGTTGCAGCATCGGGGAAAGTCCT

 431 CAGCATCAACTGATGTAGATAGTTCAACAATGGAACACGC
 431 CAGCATCAACTGATGTAGATAGTTCAACAATGGAACACGC
 429 CAGCATCAACTGATGTAGATAGTTCAACAATGGAACACGC
 514 CAGCATCAACTGATGT**C**GATAGTTCAACAATGGAACACGC

 551 CATCACTACAAC**TACA**GAAGGTGGTAAACTGGAGGAGTC
 551 CATCACTACAAC**TACA**GAAGGTGGTAAACTGGAGGAGTC
 549 CATCACTACAAC**TACA**GAAGGTGGTAAACTGGAGGAGTC
 634 CATCACTACAAC**TACA**GAAGGTGGTAAACTGGAGGAGTC

 671 TTGGTCAGAAGATTTATGAAATAGACCCCCTTTTGACAAA
 671 TTGGTCAGAAGATTTATGAAATAGACCCCCTTTTGACAAA
 669 TTGGTCAGAAGATTTATGAAATAGACCCCCTTTTGACAAA
 754 TTGGTCAGAAGATTTATGAAATAGACCCCCTTTTGACAAA

 791 AAGC**T**TTTTCTCGTGGTTATGAAAAAATGGGTTTCACTCG
 791 AAGC**T**TTTTCTCGTGGTTATGAAAAAATGGGTTTCACTCG
 789 AAGCTTTTTCTCGTGGTTATGAAA**G**AATGGGTTTCACTCG
 874 AAGCTTTTTCTCGTGGTTATGAAAAAATGGGTTTCACTCG

Fig.8
Sheet 2

Fig.8 SHEET 1

SUBSTITUTE SHEET (RULE 26)

26/75

ACTCCTATCACTTATCAGATCTCTATTT 11con.seq
ACTCCTATCACTTATCAGATCTCTATTT 19con.seq
ACTG~~CC~~ATCACTTATCAGATCTCTATTT 10con.seq
ACTCCTATCACT~~G~~ATCAGATCTCTATTT psbe2con.seq

GGAGTTCGTTTTCTACTGTTCCATCAG 11con.seq
GGAGTTCGTTTTCTACTGTTCCATCAG 19con.seq
GGAGTTCGTTTTCTACTGTTCCATCAG 10con.seq
GGAGTTCGTTTTCTACTGTTCCATCAG psbe2con.seq

TTGGCTGAAAAGTCTTCTTACAATTCCG 11con.seq
TTGGCTGAAAAGTCTTCTTACAATTCCG 19con.seq
TTGGCTGAAAAGTCTTCTTACAATTCCG 10con.seq
TTGGCTGAAAAGTCTTCTTAC~~G~~ATTCCG psbe2con.seq

TTCAGTCTGAGACATCTCCAGAAAATTCCC 11con.seq
TTCAGTCTGAGACATCTCCAGAAAATTCCC 19con.seq
TTC~~G~~CTGAGACATCTCCAGAAAATTCCC 10con.seq
TTCAGTCTGAGAC~~G~~CTCCAGAAAATTCCC psbe2con.seq

GGAAGTGTTGAAGAGCTGGATTTTGCTT 11con.seq
GGAAGTGTTGAAGAGCTGGATTTTGCTT 19con.seq
GGAAGTGTTGAAGAGCTGGATTTTGCTT 10con.seq
GGAAGTGTTGAAGAG~~T~~TGGATTTTGCTT psbe2con.seq

AGAGAGAGGGGCATCCCTCCACCTGGAC 11con.seq
AGAGAGAGGGGCATCCCTCCACCTGGAC 19con.seq
AGAGAGAGGGGCATCCCTCCACCTGGAC 10con.seq
AGAGAGAGGGGCATCCCTCCACCTGGAC psbe2con.seq

GCAATTGACAAGTATGAGGGTGGTTTGG 11con.seq
GCAATTGACAAGTATGAGGGTGGTTTGG 19con.seq
GCAATTGACAAGTATGAGGGTGGTTTGG 10con.seq
GCAATTGACAAGTATGAGGGTGGTTTGG psbe2con.seq

GCCCTCATTGGAGATTTCAACAATTGGG 11con.seq
GCCCTCATTGGAGATTTCAACAATTGGG 19con.seq
GCCCTCATTG~~G~~GATTTCAACAATTGGG 10con.seq
G~~T~~CTCATTGGAGATTTCAACAATTGGG psbe2con.seq

Fig. 8
SHEET 3

SUBSTITUTE SHEET (RULE 26)

28/75

TGGGAGATTTTCTGCCAAATAATGTGGATGGTTCTCCTGCAATTC
TGGGAGATTTTCTGCCAAATAATGTGGATGGTTCTCCTGCAATTC
TGAAGAGATTTTCTGCCAAATAATGTGGATGGTTCTCCTGCAATTC
TGGGAGATTTTCTGCCAAATAATGTGGATGGTTCTCCTGCAATTC

TACTCTTTACAGCTTCCTGATGAAATTCCATATAATGGAATATATT
TACTCTTTACAGCTTCCTGATGAAATTCCATATAATGGAATAATT
TACTCTTTACAGCTTCCTGATGAAATTCCATATAATGGAATATATT
TACTCTTTACAGCTTCCTGATGAAATTCCATATAATGGAATATATT

GAATCTCATATTGGAATGAGTAGTCCGGAGCCTAAAATTAACATCAT
GAATCTCATATTGGAATGAGTAGTCCGGAGCCTAAAATTAACATCAT
GAATCTCATATTGGAATGAGTAGTCCGGAGCCTAAAATTAACATCAT
GAATCTCATATTGGAATGAGTAGTCCGGAGCCTAAAATTAACATCAT

TCAAGAGCATTCTTATTATGCTAGTTTTGGTTATCATGTCACAAAT
TCAAGAGCATTCTTATTATGCTAGTTTTGGTTATCATGTCACAAAT
TCAAGAGCATTCTTATTATGCTAGTTTTGGTTATCATGTCACAAAT
TCAAGAGCATTCTTATTATGCTAGTTTTGGTTATCATGTCACAAAT

AATTGTTGTTCTCATGGACATTGTTTACAGCCATGCATCAAATAAT
AATTGTTGTTCTCATGGACATTGTTTACAGCCATGCATCAAATAAT
AATTGTTGTTCTCATGGACATTGTTTACAGCCATGCATCAAATAAT
AATTGTTGTTCTCATGGACATTGTTTACAGCCATGCATCAAATAAT

GATGTGGGATTCCGCCTCTTTAACTATGGAAACTGGGAGGTACTT
GATGTGGGATTCCGCCTCTTTAACTATGGAAACTGGGAGGTACTT
GATGTGGGATTCCGCCTCTTTAACTATGGAAACTGGGAGGTACTT
GATGTGGGATTCCGCCTCTTTAACTATGGAAACTGGGAGGTACTT

ATCAATGATGTATACTCACCACGGATTATCGGTGGGATTCACTGGG
ATCAATGATGTATACTCACCACGGATTATCGGTGGGATTCACTGGG
ATCAATGATGTATACTCACCACGGATTATCGGTGGGATTCACTGGG
ATCAATGATGTATACTCACCACGGATTATCGGTGGGATTCACTGGG

TCATAGGCTTTTCCCAGATGCAATTACCATTGGTGAAGATGTTAGC
TCATGGGCTTTTCCCAGATGCAATTACCATTGGTGAAGATGTTAGC
TCATGGGCTTTTCCCAGATGCAATTACCATTGGTGAAGATGTTAGC
TCATGGGCTTTTCCCAGATGCAATTACCATTGGTGAAGATGTTAGC

Fig. 8
Sheet 6

Fig. 8
SHEET 5

SUBSTITUTE SHEET (RULE 26)

1868 GGGGGTGTGGCTTTGACTATCGGCTGCATATGGCAATTGC
1870 GGGGGTGTGGCTTTGACTATCGGCTGCATATGGCAATTGC
1869 GGGGGTGTGGCTTTGACTATCGGCTGCATATGGCAATTGC
1953 GGGGGTGTGGCTTTGACTATCGGCTGCATATGGCAATTGC

1988 AGATGGTCGGAAAAGTGTGTTTCATACGCTGAAAGTCATGA
1990 AGATGGTCGGAAAAGTGTGTTTCATACGCTGAAAGTCATGA
1989 AGATGGTCGGAAAAGTGTGTTTCATACGCTGAAAGTCATGA
2073 AGATGGTCGGAAAAGTGTGTTTCATACGCTGAAAGTCATGA

2108 CCG**C**CAACATCATTAAATAGATCGTGGGATAGCATTGCACAA
2110 CCGTCAACATCATTAAATAGATCGTGGGATAGCATTGCACAA
2109 CCGTCAACATCATTAAATAGATCGTGGGATAGCATT**A**CACAA
2193 CCGTCAACATCATTAAATAGATCGTGGGATAGCATTGCACAA

2228 TGGATTGATTTCCCTAGGGCTGAGCCACACCTTCTGATGG
2230 TGGATTGATTTCCCTAGGGCTGAACAACACCTCTCTGATGG
2229 TGGATTGATTTCCCTAGGGCTGAACAACACCTCTCTGATGG
2313 TGGATTGATTTCCCTAGGGCTGAACAACACCTCTCTGATGG

2348 TACC**A**TGGGTT**A**CAAGAATTTGAC**T**GGGCTATGCAGTATCT
2350 TACCGTGGGTTGCAAGAATTTGACCGG**C**CTATGCAGTATCT
2349 TACCGTGGGTTGCAAGAATTTGACCGGGCTATGCAGTATCT
2433 TACCGTGGGTTGCAAGAATTTGACCGGGCTATGCAGTATCT

2468 GAAA**G**AGGAAACCTAGTTTT**G**CTTTAATTTTCACTGGAC
2470 GAAAAAGGAAACCTAGTTTTTGTCTTTAATTTTCACTGGAC
2469 GAAAAAGGAAACCTAGTTTTTGTCTTTAATTTTCACTGGAC
2553 GAAAAAGGAAACCTAGTTTTTGTCTTTAATTTTCACTGGAC

2588 TTTGGTGGCTTCGGGAGAATTGATCATAATGCCGAATATTT
2590 TTTGGTGGCTTCGGGAGAATTGATCATAATGCCGAATATTT
2589 TTTGGTGGCTTCGGGAGAATTGATCATAATGCCGAATATTT
2673 TTTGGTGGCTTCGGGAGAATTGATCATAATGCCGAATGTTT

2708 CTAGTAGACAAA**CT**AGAA**-----**
2710 CTAGTAGACAAAG**AAGAAGAAGAAGAAGAAG**AAGAAGA****
2709 CTAGTAGACAAAG**AAGAAGAAGAAGAAGAAG**-----
2793 CTAGTAGACAAAG**AAGAAGAAGAAGAAG**-----

Fig.8
Sheet 8

Fig. 8
SHEET 7

SUBSTITUTE SHEET (RULE 26)

32/75

GTGGGTGATATTGTTTCATACACTGACAAATAGA 11con.seq
GTGGGTGATATTGTTTCATACACTGACAAATAGA 19con.seq
GTGGGTGATATTGTTTCATACACTGACAAATAGA 10con.seq
GTGGGTGATATTGTTTCATACACTGACAAATAGA psbe2con.seq

AAGGATATGTATGATTTTATGGCTCTGGATAGA 11con.seq
AAGGATATGTATGATTTTATGGCTCTGGATAGA 19con.seq
AAGGATATGTATGATTTTATGGCTCTGGATAGA 10con.seq
AAGGATATGTATGATTTTATGGCTTTGGATAGA psbe2con.seq

AATTTTCATGGGAAATGAATTCGGCCACCCTGAG 11con.seq
AATTTTCATGGGAAATGAATTCGGCCACCCTGAG 19con.seq
AATTTTCATGGGAAATGAATTCGGCCACCCTGAG 10con.seq
AATTTTCATGGGAAATGAATTCGGCCACCCTGAG psbe2con.seq

AGATTTGACCTGGGAGATGCAGAATATTTAAGA 11con.seq
AGATTTGACCTGGGAGATGCAGAATATTTAAGA 19con.seq
AGATTTGACCTGGGAGATGCAGAATATTTAAGA 10con.seq
AGATTTGACCTGGGAGATGCAGAATATTTAAGA psbe2con.seq

CGAAAGGATGAAGGAGATAGGATGATTGTATTT 11con.seq
CGAAAGGATGAAGGAGATAGGATGATTGTATTT 19con.seq
CGAAAGGATGAAGGAGATAGGATGATTGTATTT 10con.seq
CGAAAGGATGAAGGAGATAGGATGATTGTATTT psbe2con.seq

TACAAGGTTGCTTGGACTCAGATGATCCACTT 11con.seq
TACAAGGTTGCCTTGGACTCAGATGATCCACTT 19con.seq
TACAAGGTTGCCTTGGACTCAGATGATCCACTT 10con.seq
TACAAGGTTGCCTTGGACTCAGATGATCCACTT psbe2con.seq

TATGCACCTAGTAGAACAGCAGTGGTCTATGCA 11con.seq
TATGCACCTTGTAAACAGCAGTGGTCTATGCA 19con.seq
TATGCACCTAGTAGAACAGCAGTGGTCTATGCA 10con.seq
TATGCACCTAGTAGAACAGCAGTGGTCTATGCA psbe2con.seq

AACTTGTGATCGCGTTGAAAGATTTGAACGTTA 11con.seq
AACTTGTGATCGCGTTGAAAGATTTGAACG--- 19con.seq
AACTTGTGATCGCGTTGAAAGATTTGAACG--- 10con.seq
AACTTGTGATCGCGTTGAAAGATTTGAACG--- psbe2con.seq

Fig. 8
SHEET 9

SUBSTITUTE SHEET (RULE 26)

34/75

ATCAGTCTTGGCGGAATTTCATGTGACAAAGGTTTGCACTT
TGCATCAGTCTTGGCGGAATTTTCATGTGACAAAGGTTTGCAATT
TGCATCAGTCTTGGCGGAATTTTCATGTGACAAAGGTTTGCAATT
TGCATCAGTCTTGGCGGAATTTTCATGTGACAAAGGTTTGCAATT

TTTATGTCGAATGCTGGGACGATCGAATTCCTGCAGCC
TTTATGTCGAATGCTGGGACGATCGAATTCCTGCAG
TTTATGTCGAATGCTGGGACGATCGAATTCCTGCAGCC
TTTATGTCGAATGCTGGGACGGCTTCAGCAGGTTTGTCTTAGTGA

Fig. 8
Sheet 12

CATAAAATGGAAATAGTGCTGATCTAATGATGTTTTAANCCNNNNA

Fig. 8 SHEET 11

SUBSTITUTE SHEET (RULE 26)

36/75

GGATGCTAATGTTTCTGTATTCTTGAAAAAGCACTCTCTTTTCACGG
CCTACGATTACAAAGACATAAGAAGCTTTTTCGTGAGAGAAAGTGCC
A N V S V F L K K H S L S R

TTCTACAGTTGCAGCATCGGGGAAAGTCCTTGTGCCTGGAAYCCAG
AAGATGTCAACGTCGTAGCCCCTTTCAGGAACACGGACCTTRGGTC
S T V A A S G K V L V P G ? Q

GACATCTCCAGAAAATTCCCCAGCATCAACTGATGTAGATAGTTCA
CTGTAGAGGTCTTTTAAGGGGTCGTAGTTGACTACATCTATCAAGT
T S P E N S P A S T D V D S S

TGAGCCGTCAAGTGATCTTACAGGAAGTGTTGAAGAGCTGGATTTT
ACTCGGCAGTTCACTAGAATGTCCTTCACAACTTCTCGACCTAAAA
E P S S D L T G S V E E L D F

TAAACATTAAATACTTCTGAAGAGACAATTATTGATGAATCTGAT
ATTTTGTAATTTATGAAGACTTCTCTGTTAATAACTACTTAGACTA
K T L N T S E E T I I D E S D

Hinc II

GATTTATGAAATAGACCCCCTTTTGACAACTATCGTCAACACCTT
CTAAATACTTTATCTGGGGGAAAGTGTGATAGCAGTTGTGGAA
I Y E I D P L L T N Y R Q H L

Fig.9
Sheet
2

Fig.9 SHEET 1

SUBSTITUTE SHEET (RULE 26)

38/75

Hind III

CAAGTATGAGGGTGGTTTGGGAAGCTTTTTCTCGTGGTTATGAAAAA
GTTCACTACTCCACCAAACCTTCGAAAAAGAGCACCAATACTTTTT
K Y E G G L E A F S R G Y E K

Pvu II

GGCTCCTGGTGCCAGTCAGCTGCCCTCATTGGAGATTTCAACAAT
CCGAGGACCACGGGTCAGTCGACGGGAGTAACCTCTAAAGTTGTTA
A P G A Q S A A L I G D F N N

CTGGGAGATTTTTCTGCCAAATAATGTGGATGGTTCTCCTGCAATT
GACCCTCTAAAAAGACGGTTTATTACACCTACCAAGAGGACGTAA
W E I F L P N N V D G S P A I

TGTTAAGGATTCCATTCCTGCTTGGATCAACTACTCTTTACAGCTT
ACAATTCCTAAGGTAAGGACGAACCTAGTTGATGAGAAATGTCGAA
V K D S I P A W I N Y S L Q L

AGAGGAGAGGTATRTCTTCCAACACCCACGGCCAAAGAAACCAAAG
TCTCCTCTCCATAYAGAAGGTTGTGGGTGCCGGTTTCTTTGGTTTC
E E R Y ? F Q H P R P K K P K

Fig.9
Sheet
4

Fig.9 SHEET 3

SUBSTITUTE SHEET (RULE 26)

40/75

Xmn I

GCCTAAAATTAAC TCATACGTGAATTTTAGAGATGAAGTTCTTCCT
CGGATTTTAATTGAGTATGCACTTAAATCTCTACTTCAAGAAGGA
P K I N S Y V N F R D E V L P

TCAAGAGCATTCTTATTATGCTAGTTTTGGTTATCATGTCACAAAT
AGTTCTCGTAAGAATAATACGATCAAAACCAATAGTACAGTGTTTA
Q E H S Y Y A S F G Y H V T N

GTCTTTGATTGATAAAGCTCATGAGCTAGGAATTGTTGTTCTCATG
CAGAACTAACTATTTTCGAGTACTCGATCCTTAACAACAAGAGTAC
S L I D K A H E L G I V V L M

GAACATGTTTGACGGCACAGATAGTTGTTACTTTCACTCTGGAGCT
CTTGTAACAACTGCCGTGTCTATCAACAATGAAAGTGAGACCTCGA
N M F D G T D S C Y F H S G A

AACTGGGAGGTACTTAGGTATCTTCTCTCAAATGCGAGATGGTGG
TTTGACCCTCCATGAATCCATAGAAGAGAGTTTACGCTCTACCACC
N W E V L R Y L L S N A R W W

ATCAATGATGTATACTCACCACGGATTATCGGTGGGATTCACTGGG
TAGTTACTACATATGAGTGGTGCCTAATAGCCACCCTAAGTGACCC
S M M Y T H H G L S V G F T G

Fig.9
Sheet
6

Fig.9 SHEET 5

SUBSTITUTE SHEET (RULE 26)

42/75

Hinc II

TGTGTATCTGATGCTGGTCAACGATCTTATTACGGGCTTTTCCCA
ACACATAGACTACGACCAGTTGCTAGAATAAGTGCCCGAAAAGGGT
V Y L M L V N D L I H G L F P

TTGTATTCCCGTTCAAGATGGGGGTGTTGGCTTTGACTATCGGCTG
AACATAAGGGCAAGTTCTACCCCCACAACCGAAACTGATAGCCGAC
C I P V Q D G G V G F D Y R L

GGATGAGGATTGGAGAGTGGGTGATATTGTTCATACACTGACAAAT
CCTACTCCTAACCTCTCACCCACTATAACAAGTATGTGACTGTTTA
D E D W R V G D I V H T L T N

TCAAGCTCTAGTCGGTGATAAACTATAGCATYCTGGCTGATGGAC
AGTTCGAGATCAGCCACTATTTTGATATCGTARGACCGACTACCTG
Q A L V G D K T I A ? W L M D

ATTAATAGATCGTGGGATAGCATTGCACAAGATGATTAGGCTTGTA
TAATTATCTAGCACCCCTATCGTAACGTGTTCTACTAATCCGAACAT
L I D R G I A L H K M I R L V

Fig.9
Sheet
8

Fig.9 SHEET 7

SUBSTITUTE SHEET (RULE 26)

44/75

EcoR I

TGAATTCGGCCACCCTGAGTGGATTGATTTCCCTAGGGCTGARCAA
 ACTTAAGCCGGTGGGACTCACCTAACTAAAGGGATCCCGACTYGT
 E F G H P E W I D F P R A E O

Ssp I

TGATAAATGCAGACGGAGATTTGACCTGGGAGATGCAGAATATTTA
 ACTATTTACGTCTGCCTCTAAACTGGACCCTCTACGTCTTATAAAT
 D K C R R R F D L G D A E Y L

TGAAGATAAATATGAGTTTATGACTTCAGAACACCAGTTCATATCA
 ACTTCTATTTATACTCAAATACTGAAGTCTTGTGGTCAAGTATAGT
 E D K Y E F M T S E H Q F I S

CCTAGTTTTTGTCTTTAATTTTCACTGGACAAATAGCTATTCAGAC
 GGATCAAAAACAGAAATTAAGTGGACCTGTTTATCGATAAGTCTG
 L V F V F N F H W T N S Y S D

GGACTCAGATGATCCACTTTTTTGGTGGCTTCGGGAGAATTGATCAT
 CCTGAGTCTACTAGGTGAAAAACACCGAAGCCCTCTTAAGTAGTA
 D S D D P L F G G F G R I D H

YCGYYCAATTATGGTGTATGCACCTAGTAGAACAGCAGTGGTCTAT
 RGCRRGTTAATACCACATACGTGGATCATCTTGTCGTCACCAGATA
 R ? I M V Y A P S R T A V V Y

NGAAGAATTTT

NCTTCTTAAAA

E E F

2531

Fig 9 SHEET 9

SUBSTITUTE SHEET (RULE 26)

Fig 9
Sheet
10

46/75

	10	20	30
1	-GATGGG	CCTTGA	ACTCAGCAATTTGACACTCAGT
1	TGATGGG	-CCTTGA	ACTCAGCAATTTGACACTCAGT
1	TGATGGG	CCTTGA	ACTCAGCAATTTGACACTCAGT
1	T-	-	-
1	-	-	-
	80	90	100
69	TTTTTCTCTTAATTCCAACCAAGG-	AATGAATAAAAA	A
70	TTTTTCTCTTAATTCCAACCA	GGGGAATGAATAAAAG	
71	TTTTTCTCTTAATTCCAACCAAGG-	AATGAATAAAAG	
7	-	-	AAGAG
1	-	-	-
	150	160	170
138	GAAAGATGGTGTATACACTCTCTGGAGTTCGTTTTCC		
140	GAAAGATGGTGTATATA	TA	CTCTCTGGAGTTCGTTTTCC
140	GAAAGATGGTGTATACACTCTCTGGAGTTCGTTTTCC		
33	-	-	TCT
1	-	-	-
	220	230	240
208	CAGCAGTAATGGTGATCGGAGGAATGCTAAT	ATTTCT	
210	CAGCAGTAATGGTGATCGGAGGAATGCTAATGTTTCT		
210	CAGCAGTAATGGTGATCGGAGGAATGCTAATGTTTCT		
48	CA	-	-
1	-	GGATGCTAATGTTTCT	
	290	300	310
278	ATCTTGGCTGAAAAGTCTTCTTACAATTCCGAAT	CCC	*
280	ATCTTGGCTGAAAAGTCTTCTTACAATTCCGAATTCC		
280	ATCTTGGCTGAAAAGTCTTCTTACAATTCCGAATTCC		
57	ATCTTGGCTGAAAAGTCTTCTTACAATTCCGAATTCC		
50	ATCTTGGCTGAAAAGTCTTCTTACAATTCCGAAT	CCC	*

Fig.10
Sheet 2

Fig. 10 SHEET 1

SUBSTITUTE SHEET (RULE 26)

48/75

	360	370	380
348	TTGTGCCTGGAAT	CCAGAGTGATAGCTCCTCATCCTC	
350	TTGTGCCTGGAACCCAGAGTGATAGCTCCTCATCCTC		
350	TTGTGCCTGGAACCCAGAGTGATAGCTCCTCATCCTC		
127	TTGTGCCTGGAACCCAGAGTGATAGCTCCTCATCCTC		
120	TTGTGCCTGGAAY	CCAGAGTGATAGCTCCTCATCCTC	
	430	440	450
418	AGAAAATTCCCCAGCATCAACTGATGTAGATAGTTCA		
420	AGAAAATTCCCCAGCATCAACTGATGTAGATAGTTCA		
420	AGAAAATTCCCCAGCATCAACTGATGTAGATAGTTCA		
197	AGAAAATTCCCCAGCATCAACTGATGTAGATAGTTCA		
190	AGAAAATTCCCCAGCATCAACTGATGTAGATAGTTCA		
	500	510	520
488	AACGATGACGTTGAGCCGTCAAGTGATCTTACAGGAA		
490	AACGATGACGTTGAGCCGTCAAGTGATCTTACAGGAA		
490	AACGATGACGTTGAGCCGTCAAGTGATCTTACAGGAA		
267	AACGATGACGTTGAGCCGTCAAGTGATCTTACAGGAA		
260	AACGATGACGTTGAGCCGTCAAGTGATCTTACAGGAA		
	570	580	590
558	AACTACAAGAAGGTGGTAAACTGGAGGAGTCTAAAC		
560	AACTACAAGAAGGTGGTAAACTGGAGGAGTCTAAAC		
560	AACTACAAGAAGGTGGTAAACTGGAGGAGTCTAAAC		
337	AACTACAAGAAGGTGGTAAACTGGAGGAGTCTAAAC		
330	AACTACAAGAAGGTGGTAAACTGGAGGAGTCTAAAC		
	640	650	660
628	ATCTGATAGGATCAGAGAGAGGGGCATCCCTCCACCT		
630	ATCTGATAGGATCAGAGAGAGGGGCATCCCTCCACCT		
630	ATCTGATAGGATCAGAGAGAGGGGCATCCCTCCACCT		
407	ATCTGATAGGATCAGAGAGAGGGGCATCCCTCCACCT		
400	ATCTGATAGGATCAGAGAGAGGGGCATCCCTCCACCT		

Fig.10
Sheet 4

Fig.10 SHEET 3

SUBSTITUTE SHEET (RULE 26)

50/75

	710	720	730
698	CTTTTGACAAACTATCGTCAACACCTTGATTACAGGT		
700	CTTTTGACAAACTATCGTCAACACCTTGATTACAGGT		
700	CTTTTGACAAACTATCGTCAACACCTTGATTACAGGT		
477	CTTTTGACAAACTATCGTCAACACCTTGATTACAGGT		
470	CTTTTGACAAACTATCGTCAACACCTTGATTACAGGT		
	780	790	800
768	ACAAGTATGAGGGTGGTTTGGGAAGCTTTTCTCGTGG		
770	ACAAGTATGAGGGTGGTTTGGGAAGC-TTTTCTCGTGG		
770	ACAAGTATGAGGGTGGTTTGGGAAGCCTTTTCTCGTGG		
547	ACAAGTATGAGGGTGGTTTGGGAAGCTTTTCTCGTGG		
540	ACAAGTATGAGGGTGGTTTGGGAAGCTTTTCTCGTGG		
	850	860	870
838	AGGTATCACTTACCGTGAGTGGGCTCCTGGTGCCCAG		
839	AGGTATCACTTACCGTGAGTGGGCTCCTGGTGCCCAG		
840	AGGTATCACTTACCGTGAGTGGGCTCCTGGTGCCCAG		
617	AGGTATCACTTACCGTGAGTGGGCTCCTGGTGCCCAG		
610	AGGTATCACTTACCGTGAGTGGGCTCCTGGTGCCCAG		
	920	930	940
908	GACGCAAATGCTGACATTATGACTCGGAATGAATTTG		
909	GACGCAAATGCTGACATTATGACTCGGAATGAATTTG		
910	GACGCAAATGCTGACATTATGACTCGGAATGAATTTG		
687	GACGCAAATGCTGACATTATGACTCGGAATGAATTTG		
680	GACGCAAATGCTGACATTATGACTCGGAATGAATTTG		
	990	1000	1010
978	ATGGTTCTCCTGCAATTCCTCATGGGTCCAGAGTGAA		
979	ATGGTTCTCCTGCAATTCCTCATGGGTCCAGAGTGAA		
980	ATGGTTCTCCTGCAATTCCTCATGGGTCCAGAGTGAA		
757	ATGGTTCTCCTGCAATTCCTCATGGGTCCAGAGTGAA		
750	ATGGTTCTCCTGCAATTCCTCATGGGTCCAGAGTGAA		

Fig.10
Sheet 6

Fig.10 SHEET 5

SUBSTITUTE SHEET (RULE 26)

52/75

	1060	1070	1080
1048	TTCCATT	CCTGCTT	GGATCAACTACTCTTTACAGCTT
1049	TTCCATT	CCTGCTT	GGATCAACTACTCTTTACAGCTT
1050	TTCCATT	CCTGCTT	GGATCAACTACTCTTTACAGCTT
827	TTCCATT	CCTGCTT	GGATCAACTACTC--TACAGCTT
820	TTCCATT	CCTGCTT	GGATCAACTACTCTTTACAGCTT
	1130	1140	1150
1118	GATCCACCCGAAGAGGAGAGGTATATCTTCCAACACC		
1119	GATCCACCCGAAGAGGAGAGGTATATCTTCCAACACC		
1120	GATCCACCCGAAGAGGAGAGGTATATCTTCCAACACC		
895	GATCCACCCGAAGAGGAGAGGTATATCTTCCAACACC		
890	GATCCACCCGAAGAGGAGAGGTAT	R	TCTTCCAACACC
	1200	1210	1220
1188	ATGAATCTCATATTGGAATGAGTAGTCCGGAGCCTAA		
1189	ATGAATCTCATATTGGAATGAGTAGTCCGGAGCCTAA		
1190	ATGAATCTCATATTGGAATGAGTAGTCCGGAGCCTAA		
965	ATGAATCTCATATTGGAATGAGTAGTCCGGAGCCTAA		
960	ATGAATCTCATATTGGAATGAGTAGTCCGGAGCCTAA		
	1270	1280	1290
1258	TCTTCCTCGCATAAAAAA	AGCTTGGGTACAATGCGCT	*
1259	TCTTCCTCGCATAAAAAA	-GCTTGGGTACAATGCGCT	
1260	TCTTCCTCGCATAAAAAA	-GCTTGGGTACAATGCGCT	
1035	TCTTCCTCGCATAAAAAA	-GCTTGGGTACAATGCGCT	
1030	TCTTCCTCGCATAAAAAA	-SCTTGGGTACAATGCGCT	*
	1340	1350	1360
1328	TGCTAGTTTTGGTTATCATGTCACAAATTTTTTTGCA		
1328	TGCTAGTTTTGGTTATCATGTCACAAATTTTTTTGCA		
1329	GCTAGTTTTGGTTATCATGTCACAAATTTTTTTGCA		
1104	TGCTAGTTTTGGTTATCATGTCACAAATTTTTTTGCA		
1099	TGCTAGTTTTGGTTATCATGTCACAAATTTTTTTGCA		

Fig.10
Sheet 8

Fig.10 SHEET 7

SUBSTITUTE SHEET (RULE 26)

54/75

	1410	1420	1430
1398	AAGTCTTTGATTGATAAAGCTCATGAGCTAGGAATTG		
1398	AAGTCTTTGATTGATAAAGCTCATGAGCTAGGAATTG		
1399	AAGTCTTTGATTGATAAAGCTCATGAGCTAGGAATTG		
1174	AAGTCTTTGATTGATAAAGCTCATGAGCTAGGAATTG		
1169	AAGTCTTTGATTGATAAAGCTCATGAGCTAGGAATTG		
	1480	1490	1500
1468	CAAATAATACTTTAGATGGACTGAACATGTTTGACGG		
1468	CAAATAATACTTTAGATGGACTGAACATGTTTGACGG		
1469	CAAATAATACTTTAGATGGACTGAACATGTTTGAC		
1244	CAAATAATACTTTAGATGGACTGAACATGTTTGACGG		
1239	CAAATAATACTTTAGATGGACTGAACATGTTTGACGG		
	1550	1560	1570
1538	TGGTTATCATTGGATGTGGGATT		
1538	TGGTTATCATTGGATGTGGGATT		
1539	TGGTTATCATTGGATGTGGGATTCCCGCCTCTTTAAC		
1314	TGGTTATCATTGGATGTGGGATTCCCGCCT		
1309	TGGTTATCATTGGATGTGGGATTCCCGCCTCTTTAAC		
	1620	1630	1640
1608	TCAAATGCGAGATGGTGGTTGGATGAGTTCAAATTTG		
1607	TCAAATGCGAGATGGTGGTTGGATGAGTTCAAATTTG		
1609	TCAAATGCGAGATGGTGGTTGGATGGTTCAAATTTG		
1384	TCAAATGCGAGATGGTGGTTGGATGAGTTCAAATTTG		
1379	TCAAATGCGAGATGGTGGTTGGATGAGTTCAAATTTG		
	1690	1700	1710
1678	TGTACTACCAACGGATTATCGGTGGGATTCACTGG		
1677	TGTATACTACCAACGGATTATCGGTGGGATTCACTGG		
1679	TGTATACTACCAACGGATTATCGGTGGGATTCACTGG		
1454	TGTATACTACCAACGGATTATCGGTGGGATTCACTGG		
1449	TGTATACTACCAACGGATTATCGGTGGGATTCACTGG		

Fig. 10
Sheet 10

Fig. 10 SHEET 9

SUBSTITUTE SHEET (RULE 26)

56/75

	1760	1770	1780
1748	TGTGGATGCTGTTGTGTATCTGATGCTGGTCAACGAT		
1747	TGTGGATGCTGTTGTGTATCTGATGCTGGTCAACGAT		
1749	TGTGGATGCTGTTGTGTATCTGATGCTGGTCAACGAT		
1524	TGTGGATGCTGTTGTGTATCTGATGCTGGTCAACGAT		
1519	TGTGGATGCTGTTGTGTATCTGATGCTGGTCAACGAT		
	1830	1840	1850
1818	ATTGGTGAAGATGTTAGCGGAATGCCGACATTTTGT		
1817	ATTGGTGAAGATGTTAGCGGAATGCCGACATTTTGT		
1819	ATTGGTGAAGATGTTAGCGGAATGCCGACATTTTGT		
1594	ATTGGTGAAGATGTTAGCGGAATGCCGACATTTTGT		
1589	ATTGGTGAAGATGTTAGCGGAATGCCGACATTTTGT		
	1900	1910	1920
1888	ATCGGCTGCATATGGCAATTGCTGATAAATGGATTGA		
1887	ATCGGCTGCATATGGCAATTGCTGATAAATGGATTGA		
1889	ATCGGCTGCATATGGCAATTGCTGATAAATGGATTGA		
1664	ATCGGCTGCATATGGCAATTGCTGATAAATGGATTGA		
1659	ATCGGCTGCATATGGCAATTGCTGATAAATGGATTGA		
	1970	1980	1990
1958	GGGTGATATTGTTTCATACACTGACAAATAGAAGATGG		
1957	GGGTGATATTGTTTCATACACTGACAAATAGAAGATGG		
1959	GGGTGATATTGTTTCATACACTGACAAATAGAAGATGG		
1734	GGGTGATATTGTTTCATACACTGACAAATAGAAGATGG		
1729	GGGTGATATTGTTTCATACACTGACAAATAGAAGATGG		
	2040	2050	2060
2028	GATCAAGCTCTAGTCGGTGATAAACTATAGCATTCT		
2027	GATCAAGCTCTAGTCGGTGATAAACTATAGCATTCT		
2029	GATCAAGCTCTAGTCGGTGATAAACTATAGCATTCT		
1804	GATCAAGCTCTAGTCGGTGATAAACTATAGCATTCT		
1799	GATCAAGCTCTAGTCGGTGATAAACTATAGCATTCT		

Fig.10
Sheet 12

Fig. 10 SHEET 11

SUBSTITUTE SHEET (RULE 26)

58/75

	2110	*	2120	2130
2098	CTCTGGATAGACCGTCAACATCATTAAATAGATCGTGG			
2097	CTCTGGATAGACCGGCAACATCATTAAATAGATCGTGG			
2099	CTCTGGATAGACCGTCAACATCATTAAATAGATCGTGG			
1874	CTCTGGATAGACCGGCAACATCATTAAATAGATCGTGG			
1869	CTCTGGATAGACCGYCAACAYCATTAAATAGATCGTGG			
	2180		2190	2200
2168	TATGGGATTAGGAGGAGAAGGGTACCTAAATTTTCATG			
2167	TATGGGATTAGGAGGAGAAGGGTACCTAAATTTTCATG			
2169	TATGGGATTAGGAGGAGAAGGGTACCTAAATTTTCATG			
1944	TATGGGATTAGGAGGAGAAGGGTACCTAAATTTTCATG			
1939	TATGGGATTAGGAGGAGAAGGGTACCTAAATTTTCATG			
	2250	*	2260	2270
2238	TTCCCTAGGGCTGAACAACACCTCTCTGATGGCTCAG			
2237	TTCCCTAGGGCTGAACACACCTTCTCTGATGGCTCAG			
2239	TTCCCTAGGGCTGAACAACACCTCTCTGATGGCTCAG			
2014	TTCCCTAGGGCTGAACAACACCTCTCTGATGACTCAG			
2009	TTCCCTAGGGCTGARCAACACCTCTCTGATGGCTCAG			
	2320		2330	2340
2308	GCAGACGGAGATTTGACCTGGGAGATGCAGAATATTT			
2307	GCAGACGGAGATTTGACCTGGGAGATGCAGAATATTT			
2309	GCAGACGGAGATTTGACCTGGGAGATGCAGAATATTT			
2084	GCAGACGGAGATTTGACCTGGGAGATGCAGAATATTT			
2079	GCAGACGGAGATTTGACCTGGGAGATGCAGAATATTT			
	2390		2400	2410
2378	TATGCAGTATCTTGAAGATAAATATGAGTTTATGACT			
2377	TATGCAGTATCTTGAAGATAAATATGAGTTTATGACT			
2379	TATGCAGTATCTTGAAGATAAATATGAGTTTATGACT			
2154	TATGCAGTATCTTGAAGATAAATATGAGTTTATGACT			
2149	TATGCAGTATCTTGAAGATAAATATGAGTTTATGACT			

Fig.10
Sheet 14

Fig.10 SHEET 13

SUBSTITUTE SHEET (RULE 26)

60/75

	2460	2470	*	2480
2448	GGAGATAGGATGATTGTATTTGAAAAAGGAAACCTAG			
2447	GGAGATAGGATGATTGTATTTGAAAAGGAAACCTAG			
2449	GGAGATAGGATGATTGTATTTGAAAAAGGAAACCTAG			
2224	GGAGATAGGATGATTGTATTTGAAAAAGGAAACCTAG			
2219	GGAGATAGGATGATTGTATTTGAAAAGGAAACCTAG			
			*	
	2530	2540		2550
2518	ATTCAGACTATCGCATAGGCTGCCTGAAGCCTGGAAA			
2517	ATTCAGACTATCGCATAGGCTGCCTGAAGCCTGGAAA			
2519	ATTCAGACTATCGCATAGCCTGCCTGAAGCCTGGAAA			
2294	ATTCAGACTATCGCATAGGCTGCCTGAAGCCTGGAAA			
2289	ATTCAGACTATCGCATAGGCTGCCTGAAGCCTGGAAA			
	2600	2610		2620
2588	TTTTGGTGGCTTCGGGAGAATTGATCATAATGCCGAA			
2587	TTTTGGTGGCTTCGGGAGAATTGATCATAATGCCGAA			
2589	TTTTGGTGGCTTCGGGAGAATTGATCATAATGCCGAA			
2364	TTTTGGTGGCTTCGGGAGAATTGATCATAATGCCGAA			
2359	TTTTGGTGGCTTCGGGAGAATTGATCATAATGCCGAA			
	2670	2680	*	2690
2658	CCTCGTTCAATTATGGTGTATGCACCTAGTAGAACAG			
2657	CCTTGTTCATTATGGTGTATGCACCTAGTAGAACAG			
2659	CCTCGTTCAATTATGGTGTATGCACCTTGTAAACAG			
2434	CCTCGTTCAATTATGGTGTATGCACCTTGTAGAACAG			
2429	CCTCGTTCAATTATGGTGTATGCACCTAGTAGAACAG			
			*	
	2740	2750		2760
2722	-----AAGAAGAAGAAGAAGAAGAAGTAGCAGTAGT			
2722	-----AGAAAGTAGCAGTAGT			
2729	AAGAAGAAGAAGAAGAAGAAGAAGAAGTAGCAGCAGT			
2501	AAGAAGAAGAAGAAGAAGAAGAAGAAGTAGCAGTAGT			
2499	NAGAAGAAGAAGAAGAN-----			

Fig. 10
Sheet 16

Fig. 10 SHEET 15

SUBSTITUTE SHEET (RULE 26)

62/75

	2810	2820	2830
2786	CTTGTGATCGCGTTGAAAGATTTGAACGC	CACATAGA	
2764	CTTGTGATCGCGTTGAAAGATTTGAACG	TTACTTGG	
2799	CTTGTGATCGCGTTGAAAGATTTGAACG	CTACATAGA	
2571	CTTGTG		
2529			

	2880	2890	2900
2856	CTTGGCGGAATTTTCATGTGACAACA	-GGTTTGCAATT	
2829	CTTGGCGGAATTTTCATGTGACAACA	AGGTTTGCA	TT
2869	CTTGGCGGAATTTTCATGTGACA	CAA	-GGTTTGCAATT
2576			
2529			

	2950	2960	2970
2925	GAGATGAAGTGCTGAACAAA	AACATATGTAAAATCGA	
2899	GAGATGAAGTGCTGAACAAA	--CATATGTAAAATCGA	
2938	GAGATGAAGTGCTGAACAAA	--CATATGTAAAATCGA	
2576			
2529			

	3020	3030
2995	CCTGCAG	-----CC
2967	CCTGCAG	-----CC
3006	CCTGCAG	GCCGGGGGACCCCTTAGTTCT
2576		
2529		

Fig.10
Sheet 18

Fig. 10 SHEET 17

SUBSTITUTE SHEET (RULE 26)

64/75

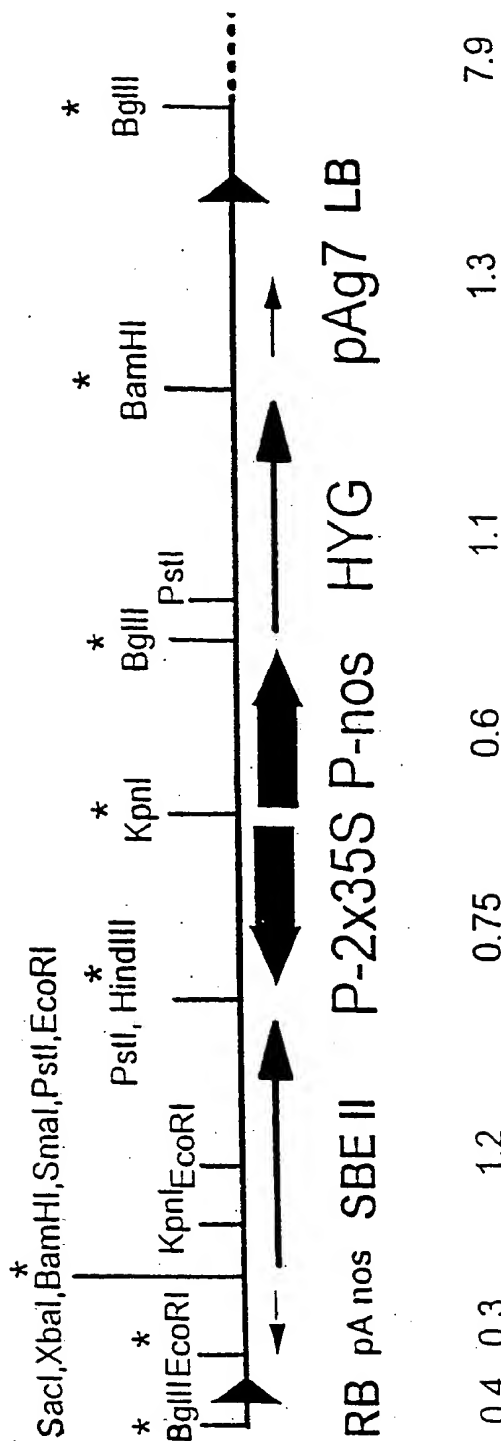


Fig. 11

66/75

Fig.12
SHEET 2

AACACGCTAGCCAGATTAAACTGAGAACGATGACGTTGAGCCGTCAAGTGATCTTACAG 300
 TTGTGCGATCGGICTAATTTTGACTCTTGCTACTGCAACTCGGCAGTTCACCTAGAATGTC
 E H A S Q I K T E N D D V E P S S D L T

 GAAGTGTGAAGAGCTGGATTTTGCTTCATCACTACAACGAGGTTGGTAAACTGG 360
 CTTCACAACTTCTCGACCTAAACGAGTAGTGTGATGTTCTTCCACCAATTGACC
 G S V E E L D F A S S L Q L Q E G G K L

 AGGAGTCTAAACATTAAATACTTCTGAAGAGACAATTATTGATGAATCTGATAGGATCA 420
 TCCTCAGATTTTGTAAATTTAAGAGACTTCTCTGTTAATAACTACTTAGACTATCCTAGT
 E E S K T L N T S E E T I I D E S D R I

 GAGAGAGGGGCATCCCTCCACCTGGACTTGGTCAGAAGATTTATGAAATAGACCCCTTT 480
 CTCCTCCCGTAGGGAGGTGGACCTGAACCAGTCTTCTAAATACTTTATCTGGGGGAAA
 R E R G I P P P G L G Q K I Y E I D P L

 Hinc II
 TGACAAACTATCGTCAACACCTTGATTACAGGTATTCACAGTACAAGAACTGAGGGAGG 540
 ACTGTTTGATAGCAGTTGTGGAACCTAATGTCCATAAGTGTCATGTTCTTTGACTCCCTCC
 L T N Y R Q H L D Y R Y S Q Y K K L R E

SUBSTITUTE SHEET (RULE 26)

68/75

SnaBI

GGTCCAGAGTGAAGATACGTAATGGACACATCCATCAGGTGTTAAGGATTCATTCCTGCTT 840
CCAGGTCTCACTTCTATGCATACCTGTGAGGTAGTCCACAATTCCTAAGGTAAGGACGAA
G S R V K I R M D T P S G V K D S I P A

GGATCAACTACTCTTCACAGCTTCCCTGATGAAATTCATATATAATGGAATATATTAATGATC 900
CCTAGTTGATGAGAAGTGTGGAAGGACTACTTTAAGGTATATTACCTTATATAATACTAG
W I N Y S S Q L P D E I P Y N G I Y Y D

CACCCGAAGAGGAGGTATATCTTCCAACACCCACGGCCAAAGAAACCAAGTCGCTGA 960
GTGGGCTTCTCCTCTCCATATAGAAGGTGTGGTGCCGGTTTCTTTGGTTTCAGCGACT
P P E E R Y I F Q H P R P K K P K S L

GAATATAATCATCATATTGGAATGAGTAGTCCGGAGCCTAAAATTAACTCATACGTGA 1020
CTTATATACTTAGAGTATAACCTTACTCATCAGGCCCTCGGATTTTAAATTGAGTATGCACT
R I Y E S H I G M S S P E P K I N S Y V

Fig. 12
SHEET 4

SUBSTITUTE SHEET (RULE 26)

70/75

Sac I

1320
ATGGACTGAACAATGTTGACGGCACCGATAGTIGTTACTTTCACCTCIGGAGCTCGTGGTT
TACCTGACTTGTACAACACTGCCGTGGCTATCAACAATGAAAGTGAGACCTCGAGCACCAA
D G L N M F D G T D S C Y F H S G A R G

1380
ATCATGGATGIGGGATTCGCCCTTTTAACTATGGAAACTGGGAGGTACTTAGGTATC
TAGTAACCTACACCCCTAAGGGCGGAAATGATACCTTTGACCCCTCCATGAATCCATAG
Y H W M W D S R L F N Y G N W E V L R Y

1440
TTCTCTCAAATGCGAGATGGTGGTGGATGAGTTCAAAATTTGATGGATTTAGATTTGATG
AAGAGAGTTTACGCTCTACCCACCACTACTCAAGTTTAACTACCTAAATCTAAACTAC
L L S N A R W W L D E F K F D G F R F D

1500
GTGTGACATCAATGATGTATACTACCCACGGATTATCGGTGGGATTCACCTGGGAACCTACG
CACACTGTAGTTACTACATATGAGTGGTGCCTAATAGCCACCCCTAAGTGACCCCTTGATGC
G V T S M M Y T H H G L S V G F T G N Y

Fig. 12
SHEET 6

SUBSTITUTE SHEET (RULE 26)

72/75

Fig 12
SHEET 8

1860
GTCATGATCAAGCTCTAGTCGGTGATAAAACTATAGCATTTCTGGCTGATGGACAAGGATA
CAGTACTAGTTCGAGATCAGCCACTATTTTGATATCGTAAGACCGACTACCTGTTTCCTAT
S H D Q A L V G D K T I A F W L M D K D

1920
TGTATGATTTTATGGCTCTGGATAGACCGCCAACATCATTAATAGATCGTGGGATAGCAT
ACATACTAAATACCGAGACCTATCTGCGGTGTAGTAATTATCTAGCACCCCTATCGTA
M Y D F M A L D R P P T S L I D R G I A

Asp 718

Kpn I

1980
TGCACAAGATGATTAGGCTTGTAACATAIGGGATTAGGAGGAGAAGGTACCTAAATTCA
ACGTGTTCTACTAATCCGAACATTGATACCCCTAATCCCTCCTCCCATGGATTAAAGT
L H K M I R L V T M G L G G E G Y L N F

EcoR I

2040
TGGGAAATGAATTCGGCCACCCIGAGTGGATTGATTCCCTAGGGCTGAACAACACCTCT
ACCCTTTACTTAAGCCGGTGGGACTCACCTAACTAAAGGGATCCCGACTTGTGTGGAGA
M G N E F G H P E W I D F P R A E Q H L

SUBSTITUTE SHEET (RULE 26)

TTGGCC TTGGACTCAGATGATCCACTTTTGGTGGCTTCGGGAGAA TTGATCATAATGCCG
AACGGAACCTGAGTCTACTAGGTGAAAAACCACCGAGCCCTCTTAAC TAGTATTACGGC
V A L D S D D P L F G G F G R I D H N A 2400

Sp1

AATAATTTACACCTTTGAAGGATGGTAATGATGATCGTCCTCGTTCAATTATGGGTATGCAC
 TTATAAAGTGGAACTTCCTACCATACTACTAGCAGGAGCAAGTTAATACCACATACGTG
 E Y F T F E G W Y D D R P R S I M V Y A

CTTGTAGAACAGCAGTGCTCTATGCACTAGTAGACAAAGAAGAAGAAGAAGAAG
GAACATCTTGTGTCACCAGATACGTGATCATCGTTCTTCTTCTTCTTCTTCTTC
P C R T A V V Y A L V D K E E E E E

AAGAAGTAGCAGTAGTAGAAGTAGTAGAAGAAGATGAACGAACTTGTG
TTCTTCATCGTCATCATCTTCTTCATCATCTTCTTACTTGCTTGAACAC
E E E V A V V E E V V E E E

2578

Fig 12
SHEET 10



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ :C12N 15/82, 15/54, C08B 30/04, A01H
5/00

A3

(11) International Publication Number:

WO 96/34968

(43) International Publication Date:

7 November 1996 (07.11.96)

(21) International Application Number: PCT/GB96/01075

(22) International Filing Date: 3 May 1996 (03.05.96)

(30) Priority Data:

9509229.2	5 May 1995 (05.05.95)	GB
9607409.1	10 April 1996 (10.04.96)	GB

(71) Applicant (for all designated States except US): NATIONAL STARCH AND CHEMICAL INVESTMENT HOLDING CORPORATION [US/US]; Suite 27, 501 Silverside Road, Wilmington, DE 19809 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): COOKE, David [GB/GB]; 2 Hunts Path, Oakley, Bedfordshire MK43 7SR (GB). DEBET, Martine [FR/GB]; 12 Bentley Close, Rectory Farm, Northampton NN3 5JS (GB). GIDLEY, Michael, John [GB/GB]; 45 Holmes Avenue, Raunds, Northamptonshire NN9 6SZ (GB). JOBLING, Stephen, Alan [GB/GB]; 19 Burwell Road, Eaton Socon, Huntingdon PE19 3QQ (GB). SAFFORD, Richard [GB/GB]; 10 Furness Close, Bedford, Bedfordshire MK41 8RN (GB). SIDEBOTTOM, Christopher, Michael [GB/GB]; 35 Waterloo Road, Bedford, Bedfordshire MK40 3PQ (GB). WESTCOTT, Roger, John

[GB/GB]; 46 Castle Street, Wellingborough, Northamptonshire NN8 1LW (GB).

(74) Agent: KEITH W. NASH & CO.; 90-92 Regent Street, Cambridge CB2 1DP (GB).

(81) Designated States: AU, BR, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

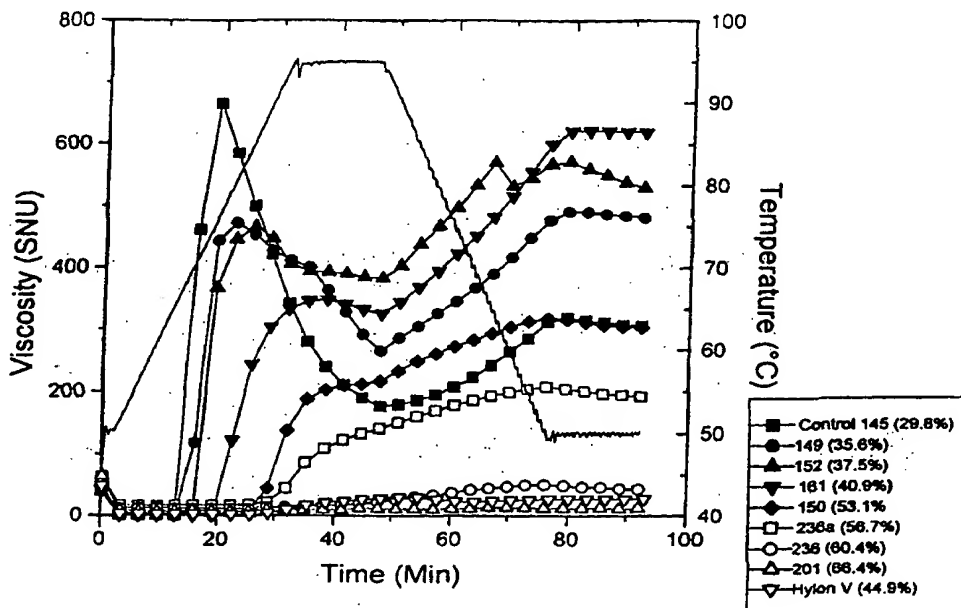
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:

5 December 1996 (05.12.96)

(54) Title: IMPROVEMENTS IN OR RELATING TO PLANT STARCH COMPOSITION



(57) Abstract

Disclosed is a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants, or a functional equivalent thereof, together with, inter alia, a corresponding polypeptide, a method of altering the characteristics of a plant, a plant having altered characteristics; and starch, particularly starch obtained from a potato plant, having novel properties.

INTERNATIONAL SEARCH REPORT

National Application No

PC:/GB 96/01075

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N15/54 C08B30/04 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C08B A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE PLANT JOURNAL, vol. 7, no. 1, January 1995, pages 3-15, XP002014042 BURTON, R.A., ET AL.: "Starch branching enzymes belonging to distinct enzyme families are differentially expressed during pea embryo development" see the whole document ---	28-32, 34,39-41
X	DATABASE WPI Section Ch, Week 9442 Derwent Publications Ltd., London, GB; Class C06, AN 94-337418 XP002014047 & JP,A,06 261 767 (MITSUI GYOSAI SHOKUBUTSU BIO KENKYUSHO) , 20 September 1994 see abstract --- -/-	28-37, 39-42, 48-50

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

Date of the actual completion of the international search

23 September 1996

Date of mailing of the international search report

23. 10. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Maddox, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 96/01075

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PLANT PHYSIOLOGY, vol. 105, no. 1, 1 May 1994, page 37 XP000561226 KROHN B M ET AL: "MODIFICATION OF STARCH STRUCTURE IN TRANSGENIC POTATO" see abstract 145 ---	27-41
A	WO,A,90 12084 (DNA PLANT TECHN CORP) 18 October 1990 see page 9, line 17 ---	44
A	ABSTRACTS VIITH INTERBATIONAL CONGRESS ON PLANT TISSUE AND CELL CULTURE, AMSTERDAM, JUNE 24-29, 1990, ABSTRACT NO. A5-28, page 177 XP002014046 VAN DER LEIJ, F.R., ET AL.: "Expression of the gene encoding granule bound starch synthase after introduction in an amylose-free and a wildtype potato (Solanum tuberosum)" see the whole document ---	1-26
A	WO,A,94 24292 (DANISCO ;VILLAND PER (NO); KLECZKOWSKI LESZEK (NO); OLSEN ODD ARNE) 27 October 1994 see page 29, line 25 - page 30, line 30 ---	40
E	WO,A,96 19581 (INST GENBIOLOGISCHE FORSCHUNG ;KOSSMANN JENS (DE); EMMERMANN MICHA) 27 June 1996 see the whole document -----	7,17, 23-26, 64,65